

Conference Report

# Abstracts of the 3rd International Electronic Conference on Diversity: Biodiversity of Animals, Plants and Microorganisms <sup>†</sup>

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**Abstract:** The 3rd International Electronic Conference on Diversity: Biodiversity of Animals, Plants and Microorganisms (IECD 2024) was held from 15 to 17 October 2024. The conference focused on the key theme of biodiversity from a wide range of domains, especially animals and plants. The conference was in a virtual format, eliminating the need for travel and other related expenses, allowing attendees to fully immerse themselves in the conference without any logistical constraints. It aimed to foster collaboration and knowledge exchange among researchers.

**Keywords:** biodiversity; animal diversity; plant diversity; microbial diversity

## 1. Animal Diversity

### 1.1. The European Red Squirrel (*Sciurus vulgaris*) Population Declined Due to Squirrelpox Brought by the Grey Squirrel (*Sciurus carolinensis*) Invasion

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The squirrelpox virus (SQPV) is a poxvirus that affects squirrel species. The affected species are the European red squirrel (*Sciurus vulgaris*), the American red squirrel (*Tamiasciurus hudsonicus*), and the Eastern grey squirrel (*Sciurus carolinensis*). *S. carolinensis* acts as a reservoir of SQPV but does not display clinical signs of the disease and is responsible for its transmission to native squirrels in Europe. The first grey squirrels were introduced in England in 1876. The first confirmed case of SQPV in Europe was in East Anglia in the 1980s, and since then, the virus has spread to other regions of the UK and other European countries. In *S. vulgaris*, the disease is characterized by multifocal skin fibroma (tumors) and ulcerative and exudative dermatitis with hemorrhagic scabs. Animals became lethargic, emaciated, and eventually die. Currently, SQPV has only been detected in red squirrel populations that have contact with grey squirrels. SQPV is believed to be transmitted horizontally through direct contact between infected and susceptible squirrels, through contact with the infected lesions or contaminated crusts. The virus can also be transmitted indirectly through environmental contamination, such as contaminated feeders, surfaces, and mosquitos. In the UK, grey squirrels have been able to spread 17 to 25 times faster through the competitive exclusion of the red squirrel due to the increased mortality of them from the SQPV. At present, vaccines have been developed against this virus, but further work is required to develop a method of administering an oral vaccine to red squirrels in the wild to stop this disease and the decline of the population.



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### 1.2. The Genus *Hydra* (Cnidaria: Hydrozoa) from São Paulo State, Brazil

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Brazil is one of the two South American countries to have records of the four genera of freshwater cnidarians, being pioneers in the study of these organisms. Nevertheless, these studies were discontinued, resulting in a significant lack of information about these organisms. *Hydra* is the best known of these genera, and from the first report in Brazil to the present, it has been in four records with somewhat detailed morphological descriptions. Three of these records describe species endemic to Brazil: *Hydra iheringi*, *Hydra salmacidis*, and *Hydra intermedia*. However, these findings are outdated and very isolated, and they require immediate taxonomic corroboration. To achieve this objective, a sampling plan was implemented between April and June 2023 in seven freshwater bodies in São Paulo State, which also included a sample from Mafra, Santa Catarina State.

*Hydra* specimens were found to adhere to different species of floating and submerged vegetation. All polyps were morphologically described in detail, their cnidocysts and sexual structures were measured and typed, and the cytochrome oxidase I (COI) marker was extracted, amplified, and purified using a protocol that was applied for subsequent sequencing. Morphological and molecular analyses indicated the presence of two species: the cosmopolitan *Hydra vulgaris* and the green species *Hydra sinensis*, which were first described in China. This is the first time that both species were described in Brazil, based on more exhaustive identification methodologies.

These results are the first of this type obtained for Brazil, and they revealed that *Hydra* is a frequent and abundant genus in the freshwater fauna from this study region. Continuing these analyses in other states would provide valuable data for the phylogeny of the group and the possibility of discovering new species. Considering that the presence or absence of these organisms reflects the conservation status of the ecosystem and that they are also bioindicators of hydrographic conditions, our studies are a valuable tool for the sustainable management of continental aquatic ecosystems.

### 1.3. *Microlestes maurus cordatulus* (Reitter, 1901) (Insecta: Coleoptera: Carabidae) Enters Bulgarian Fauna

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Bulgaria is one of the smaller European countries, but it has great biological richness and diversity. It is generally believed that many thermophilic and drought-loving Mediterranean animals live in the southern regions of the country and on the Black Sea coast. This study was a part of a project researching faunal complexes along three large river valleys and the Black Sea coast, where Mediterranean influence can be detected and which serve as main roads for the entry of thermophilic biota. The aim of this zoocenosis monitoring is to detect any changes in the composition of Bulgarian fauna. The first indication so far is the registration of *Microlestes maurus cordatulus* (Reitter, 1901) (Coleoptera: Carabidae). In

April 2023, one male specimen of this subspecies was collected in an MSS trap, situated less than 20 km from the southernmost point of the Bulgarian coast: N of Ahtopol, N 42°06'33", E 27°55'02", 9 m a.s.l. *Microlestes maurus* Sturm, 1827 is a small (2.2 to 2.8 mm) black ground beetle with short, truncate elytra and bulging eyes with short temples. Its main identification characteristics are its genitals, as its penis is sclerotized and has a distinct apex with a hook-shaped denticle. In Bulgaria, this species is widespread in the plains and foothills. It is distributed across Europe (except in the north), Caucasus, and Anterior Asia. According to the last edition of the Catalogue of the Palaearctic Coleoptera, the subspecies *M. m. cordatulus* is known only in Greece, Iran, Lebanon, and Syria. The two subspecies of *M. maurus* clearly differ by the shape of their aedeagus, with that of *M. m. cordatulus* having a more elongated apex with a significantly less salient hook than that in the Bulgarian specimen. This new location suggests that *M. m. cordatulus* most certainly preside on the Turkish Black Sea coast as well.

#### 1.4. What We Know About *Deroceras laeve* (Müller, 1774) (Gastropoda, Pulmonata): An Integrative Study of a Species Complex

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The slugs of the genus *Deroceras* (Mollusca, Gastropoda, Agriolimacidae) include more than one hundred species. One of them, *D. laeve*, has a worldwide distribution, a fact that could be related to its biology and life history. This slug has economic and sanitary importance, as it is an intermediate host of parasitic nematodes, causes crop losses, and can also affect the native fauna. In this study, we carried out a phylogenetic and phylogeographic study of *D. laeve* based on the cytochrome oxidase subunit I marker (COI), considering specimens collected in Buenos Aires (Argentina) and sequences deposited on online platforms. This information was integrated with anatomical dissections of the radulae, maxillae, and genitalia. As a result, we re-identified other species of *Deroceras*, found that *D. laeve* consists of a species complex of at least six operational taxonomic units (OTUs), and statistically showed that COI can be regarded as a good marker that delimits species within the *D. laeve* complex. In addition, possible distribution pathways of the most diversified OTU were discussed, and previous anatomical studies and radular formulas were compared with those found in the present study. In order to contribute to the knowledge of the *D. laeve* complex, we encourage further studies integrating molecular, anatomical, and ecological results.

#### 1.5. Cryptic Species of *Bombus lucorum*-Complex in Northern European Russia

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The cryptic bumblebee species of *Bombus lucorum*-complex are one of the challenges for conservation management. They are morphologically indistinguishable in most of their range, which, in most cases, leads to misidentification. Among the regions of Northern Europe, reliable data on their distribution exist in Britain, Ireland, and Finland. In this paper, we studied the distribution of the cryptic species of *B. lucorum*-complex, i.e., *B. terrestris* (Linnaeus, 1758), *B. lucorum* (Linnaeus, 1761), and *B. cryptarum* (Fabricius, 1775) in Northern European Russia. The species *B. lucorum*-complex are among the most widely distributed in Northern Europe. There are some data for the Kola Peninsula, whereby DNA barcoding confirmed the presence of only *B. cryptarum* among this complex. In general,

there is no information on the distribution of the species of *B. lucorum*-complex in Northern European Russia.

We studied over 300 specimens. Morphological characters were used for initial species identification. After that, we used the PRC-RFLP method. For the verification of this method, the prepared samples were transferred for molecular analysis. *B. cryptarum* was found in the northern and southern parts of the Murmansk Region. *B. lucorum* was recorded only in the southern part of this region. In Karelia, the higher the proportion of *B. lucorum* in the samples is in the southern part of the region. Among the species of *Bombus lucorum*-complex, three species were found in the Arkhangelsk Region, i.e., *B. cryptarum*, *B. lucorum*, and *B. terrestris*. Only *B. cryptarum* is recorded in the extreme northern localities of this region. *B. terrestris* is found only in the south-east of the Arkhangelsk Region. This pattern is generally consistent with what is known for Finland, i.e., *B. cryptarum* are dominant among the species of *B. lucorum*-complex in bumblebee communities in Northern Fennoscandia.

#### 1.6. Digital Morphological Descriptions of All Reptile Species

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More than 14,000 species and subspecies of reptiles are described. The Reptile Database now provides descriptions for about 10,000 species from the primary and secondary literature. These morphological descriptions are currently supplemented by live photographs of more than 6000 species and a pilot project for a reference library of 16,000 high-resolution images representing more than 1200 species (preserved specimens). Online links to GenBank for ~8700 species and to IUCN for ~10,000 species provide DNA sequences and range maps for genetic and geographic details, respectively.

Since full-text descriptions have limited practical value, we are working to convert textual descriptions of species into a structured database of traits so that characters can also be compared and analyzed more systematically. When integrated with geographic range maps, species identifications can be substantially simplified and also made available for non-experts. We will show examples of how these trait tables, images, extracted color patterns, etc., can be used to identify and describe reptiles on a large scale.

In combination with phylogenetic trees, our dataset will allow detailed macro-ecological studies and other biological studies [1,2].

#### 1.7. Preliminary Study of the Myrmecofauna of El Impenetrable National Park (Chaco, Argentina)

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In the Chaco region, we can find one of the largest protected areas of northern Argentina, the “El Impenetrable” National Park, with a surface close to 130,000 ha. It provides multiple habitats that sustain notable plant and animal diversity, including formicid assemblages. Ants are the most abundant arthropods in tropical forests and represent an important part of ecosystems due to them providing important ecosystemic services. They are excellent bioindicators of anthropogenic perturbations; their diversity in tropical forests is associated with the forest’s canopy coverage. Despite this, no published studies of formicid diversity in “El Impenetrable” National Park evaluate canopy coverage in this important protected area of the subtropical region. A formicid survey was conducted following the recommendations of the ALL protocol, developed as a fast and standardized way of surveying ant populations in forests. True diversity (Hill numbers) for each forest and  $\beta$ -diversity between forests (Sørensen index) were assessed, and rank-abundance curves were constructed. D0 and D1 showed that diversity is positively correlated with coverage increase. Rank-abundance curves indicated higher dominance in forests with high and intermediate coverage, while low-coverage ones exhibited a more even abundance

distribution. The  $\beta$ SOR indexes were higher than 50%, suggesting differences between the ant assemblages of each forest, with species turnover being the larger contributor (46%). We can conclude that canopy coverage directly influences the richness and diversity of ants.

### 1.8. Diversity of Helminths in Reindeer (*Rangifer tarandus*) in Russia

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A large-scale survey of helminths in reindeer (*Rangifer tarandus*) in Russia was initiated by the author in 2018. A previous similar study was conducted in 1930–1960 by K. I. Skrjabin, V. Yu. Mizkewich, and other Soviet scientists. Climate change, human expansion in the Arctic region, and animal introductions and translocations might have intensified the presence of helminth fauna in reindeer.

More than 500 fecal samples of wild and semi-wild reindeer were collected throughout Russia from Murmansk Oblast in the West to Chukotka in the East and from Franz Josef Land in the North to Buryatia and Altai in the South. This research covered nature reserves, agricultural units, and all the zoos in Russia. When possible, autopsies were also performed. In total, about 200 reindeer were examined post mortem. Fecal samples were processed via larvoscopic and ovosopic (flotation and sedimentation) techniques, along with fecal examination (in order to find macrohelminths). DNA analyses were made of adult worms and larvae primarily by targeting the internal transcribed spacer region.

The diversity of helminths found via fecal examination is represented by *Fasciola hepatica*; *Paramphistomum* spp.; *Dicrocoelium* spp.; *Moniezia expansa*; *Moniezia* spp.; small strongylids, including *Ostertagia gruehneri* (major morph) and *O. arctica* (minor morph), *O. leptospicularis*, and *O. ostertagi*; *Nematodirus* spp.; *Nematodirella longissimespiculata*; *Dictyocaulus* spp.; *Elaphostrongylus rangiferi*; *Orthostrongylus* spp.; *Varestrongylus eleguneniensis*; *Trichuris* spp.; *Capillaria* spp.; *Ascaris mosgovoyi*; and *Skrjabinema tarandi*. The helminths recovered via autopsy were *Paramphistomum* spp.; *Dicrocoelium chinensis*; *Oesophagostomum*; *Dictyocaulus* spp.; *E. rangiferi*; *Trichuris discolor*; *Setaria* spp.; *Onchocerca flexuosa*; *Onchocerca* spp.; *Echinococcus* spp.; and *Taenia* spp.

The following helminths were reported for reindeer in Russia for the first time: *Orthostrongylus* spp., *Varestrongylus eleguneniensis*, *Oesophagostomum* spp., and *T. discolor*. The *Orthostrongylus* spp. and *T. discolor* nematodes were reported for the first time for reindeer on a global scale.

### 1.9. Germplasm Characterization and Conservation of the Silkworm, *Bombyx mori*, in a Changing Climate

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Characterization is a crucial step in germplasm conservation, as it reveals important information on the traits of the genetic resources, ensuring their maximum utilization by the end users and justifying conservation efforts. Under the increasing effects of climate change, germplasm characterization is gaining prominence, as it is essential to identify the underutilized resources that are potentially resilient to changing environmental conditions. Silkworm, *Bombyx mori*, an economically beneficial Lepidopteran insect utilized for the production of silk, forms an important part of the agro-ecosystem. The insect, being highly sensitive to external environments, faces a huge threat in the future. The Central Sericultural Germplasm Resources Centre, India, plays an important role of systematically conserving 490 *Bombyx mori* genetic resources. It comprises a broad genetic base, which is necessary to cope with climate change and maintain silk productivity. This paper discussed the steps taken towards the characterization and evaluation of genetic silkworm resources for unique traits, with an emphasis on their adaptability and tolerance to biotic and abiotic stress through observation, as well as use of molecular tools. The challenges and future strategies

of ex situ germplasm conservation through modern techniques like cryopreservation and DNA banks, as well as expanding the genetic base of the collection, are explored as future courses of action.

#### 1.10. Phylogenetic Insights and Species Delimitation of *Eutyphoeus* Earthworms in India Using Integrative Taxonomy

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*Eutyphoeus* Michaelsen 1900, a native genus of Indian earthworms, was recently reclassified into the Acanthodrilidae family (previously Octochaetidae) [3]. This genus has endemic species in various regions, including Myanmar, the Eastern Himalaya, the Indo-Gangetic lowlands, and the Northeast mountains. Phylogenetic research has revealed genuine connections between these taxa. To date, only three studies—two from Mizoram and one from Meghalaya—have utilized molecular systematics for these taxa. This study employed integrative taxonomy to explore the relationships among *Eutyphoeus* species in India. This made it easier to discriminate between closely related species using mitochondrial cytochrome oxidase I (CO1). A total of 48 CO1 sequences were analyzed alongside sequences retrieved from databases using various species delimitation tools (ASAP and ABGD), phylogenetic analysis (ML and BI), and haplotype networking. The findings documented 19 *Eutyphoeus* species with CO1 sequences from two biogeographic zones, the Northeast region and the Gangetic plains (5 species of the present study), out of the 28 species reported from India. All species, except one, form monophyletic clades with strong statistical support. However, *E. incommodus* split into three lineages, suggesting the existence of cryptic species. Although *Eutyphoeus* exhibited erratic dispersal patterns, phylogeographic pattern analysis indicated that it is primarily native to its regions of origin. Additionally, this study supported Gates' (1972) [4] hypothesis on the phylogeographic distribution pattern of the genus *Eutyphoeus*.

#### 1.11. Modeling the Spatial Distribution and Habitat Suitability of *Fasciola hepatica*, a Trematode Parasite of Domestic Animals in Ukraine

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Fasciolosis is a major problem in many parts of the world, including Ukraine. The disease is also a major economic burden, causing an estimated USD 3.2 billion yearly loss to the livestock industry. Today, the spread of fasciolosis is exacerbated by a number of factors, including climate change. For the planning of successful interventions against animal fasciolosis and to target populations living in high-risk areas, it is important to be able to determine the current spatial distribution of infection. In this respect, ecological niche modeling is a popular tool to examine the ecological and spatial limitations of species; however, it is not yet widely applied to parasites due to challenges in obtaining good and meaningful occurrence data. Using a recently compiled dataset (GBIF, <https://www.gbif.org/occurrence/download/0092653-240229165702484>, accessed on 6 August 2024) consisting of 335 records of *Fasciola hepatica* of domestic animals in Ukraine, we predicted the nationwide spatial distribution and habitat suitability of the fluke and responses to climate. Climate data were retrieved from the Chelsa project (<https://chelsa-climate.org/bioclim>, accessed on 6 August 2024). The 'flexsdm' R package (<https://sjevelazco.github.io/flexsdm>, accessed on 6 August 2024) was used for mapping the potential geographic distribution of the parasite across the country. A range of modeling machine learning options were tested. Eventually, an ensemble model was created based on the weighted average of the individual models, and established evaluation metrics showed good performance: AUC = 0.82, TSS = 0.58, Boyce index = 0.86. In terms of high-risk



areas, the top five provinces are Ivano-Frankiv's'k, Vinnytsya, Ternopil', L'viv, and Chernivtsi. A SHAP library in R (<https://github.com/pablo14>, accessed on 6 August 2024) was used to understand the importance in the trained model of the climatic variables. SHAP offers advantages over other feature importance methods, including its model-agnostic nature. By calculating the average absolute values for each feature, the strongest influence on predictions had isothermality, followed by an intricate balance of temperatures and sufficient precipitation.

#### 1.12. The Evaluation of a Citizen Science Project on Birdwatching in Germany

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Since 2005, The Nature And Biodiversity Conservation Union (NABU, Germany) has called people in Germany to count birds in their gardens during a defined time (Friday to Sunday) in May. Similar to the Big Garden Birdwatch in Great Britain, which has been performed since 1979, the German "Stunde der Gartenvögel" (hour of the garden birds) aims to conduct a yearly overview of the birds in villages and towns. While this birdwatching action allowed for a broad overview, due to the large number of gardens and parks involved (around 40,000 in the year 2024), several drawbacks have to be mentioned that may impede the comparability of the counted bird numbers from one year to another. The number of people counting changes, and the weather influences the results. General problems in all years are the erroneous identifications of birds, birds not mentioned since they could not be identified, or the typical focus on the daytime, so that nocturnal bird species are mostly ignored. Here, we provided an overview of the chronological development of the overall numbers of species, specifically the most frequent garden birds. We discussed correlations with external factors, such as weather and number of participants, and compared the results with ornithological studies in the same period as well as with the results of the yearly Birdrace in Germany, where ornithologically interested people count birds in a defined area on the first Saturday in May.

#### 1.13. New Cytogenetic Data of *Artibeus (Dermanura) glaucus* (Chiroptera, Phyllostomidae) and Expansion of Geographic Limits for Brazil

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Four species of the subgenus *Dermanura*, genus *Artibeus*, are known to reside in Brazil (*A. anderseni*, *A. bogotensis*, *A. cinereus*, and *A. gnoma*), with two of them (*A. anderseni* and *A. gnoma*) found in sympatry in the central–western region. *Artibeus (Dermanura) glaucus* is a small frugivorous bat that predominantly inhabits forested areas and has a neotropical distribution, found in Venezuela, Colombia, Peru, and Bolivia. The aim of this study is to characterize the cytogenetics of *A. glaucus* to understand the chromosomal diversity within the subgenus *Dermanura* and to observe chromosomal characteristics that may aid in the cytotaxonomy of the group for Brazil. This is important given the overlapping distribution limits of congeneric species and the cryptic nature of the taxon, which make

identification a challenging task based solely on morphology. A female specimen of *A. glaucus* was collected in Parque Estatal Serra Ricardo Franco, municipality of Vila Bela de Santíssima Trindade, state of Mato Grosso, Brazil. The specimen was identified through teeth and external characteristics based on the literature. Chromosomal preparations were obtained from bone marrow cells after in vivo treatment with colchicine and primary culture of fibroblasts. Metaphase cells were analyzed using Giemsa staining and G- and C-banding. We describe for the first time the karyotype of a Brazilian specimen of *A. glaucus* as  $2n = 30$  and  $Nfa = 56$ . The karyotype showed 14 meta/submetacentric pairs and a metacentric X chromosome. The literature data confirmed the same  $2n$  and  $NFa$  for a specimen from Peru. Cytogenetic data on *Dermanura* indicated chromosomal conservatism; however, molecular cytogenetic analysis using repetitive DNA probes may provide useful characteristics for better comparisons among species of the subgenus. These findings may support conservation guidelines and improve knowledge on its evolutionary history and biodiversity.

#### 1.14. Standard Metabolic Rate and Daily Activity Patterns of *Australoheros facetus* (Jenyns, 1842)

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We provide first-time estimates of the standard metabolic rates (SMRs) of *Australoheros facetus*, a freshwater cichlid fish that lives in streams and lakes in South America. Individuals of *A. facetus* were sourced from the Naposta Grande river, located in southern Buenos Aires province, Argentina, during spring (September–November in the southern hemisphere) of 2023. The individuals of *A. facetus* were captured using traps, baited, and set close to submerged vegetation on the margins of the river at shallow depths. The traps were left undisturbed for up to 45 min. The traps were then pulled up by hand, and the individuals were collected and transferred to aerated transportation tanks. The fish were allowed to recover in these acclimation tanks from the stress induced by capture and transport for 1 month. Experiments were conducted following the guidelines established by the EU Directive 2010/63/EU and the Argentinean Law n° 14346 on “The protection of animals”, as well as recommendations of the institutional committee for the use and care of laboratory animals (CICUAL). We quantified SMR and aerobic scope and daily activity patterns, finding this fish to be active during the day and sluggish during the night. Its metabolic rate is within the ranges expected for cichlids (SMR:  $104.48 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) and increases slightly during daylight hours ( $126.25 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ). Having information on the metabolic rates and aerobic scope of this species provides another piece to the puzzle of the biology of *A. facetus*, complementing the research on oxidative stress and social behavior that has already been published and future research efforts. Furthermore, it constitutes progress towards the development of predictive models on the effects of increasing water temperatures in a global warming scenario.

#### 1.15. Non-Invasive Methods to Determine Biodiversity Using Plant Gall Volume and Insect Feces

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The vast majority of methods for insect biodiversity assessment are invasive and require insect specimen collection and fixation. Specifically, the current methods are unsuitable for studying insect communities that reside in plant galls, which serve as intricate habitats for various organisms, including hosts, predators, parasitoids, and inquilines. In this research, we proposed new non-invasive methods for the quantitative and qualitative determination of insect biodiversity in plant galls. A gall is a mini ecosystem consisting of



gall-formers, their parasitoids, predators, and inquilines. The main challenge in the determination of insect species in a gall is the need to dissect a gall, destroying a hidden ecosystem. The development of non-invasive methods will allow for the study of endangered insect species inside galls. Using regression analysis, we established a precise correlation between the volume of *Hieracium robustum* Fr. galls and the number of its inhabitants, as well as the ratio of hosts to parasitoids. Moreover, we found that each of the nine insect species in these galls has a unique morphological pattern of juvenile feces. Therefore, the species composition can be determined using these morphological observations. Importantly, this method is non-invasive, as the feces observation inside the galls occurs after the emergence of gall inhabitants in their natural environment and seasonal plant death. Our research can pave the way for new methods in biodiversity measurements, specifically in plant galls.

#### 1.16. Investigation of Cotton Fabric Coating Using Fungal Mycelium *Pleurotus ostreatus* and Beeswax

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The sustainable use of bio-based materials is essential as they promote resource efficiency, minimize environmental impact, and ensure the longevity and health of ecosystems and human populations. Mushroom mycelium, known for its antibacterial, anti-inflammatory, and antiviral properties, and beeswax, known for its water resistance, pleasant odor, and antibacterial properties, are examples of sustainable materials that contribute to sustainable goals. In this study, the coating of cotton fabrics with a wax mixture containing fungal mycelium was investigated. The aim of the research was to evaluate the improvement in textile properties such as water resistance, abrasion resistance, knitter resistance, and other mechanical properties. Comparative analyses were carried out on uncoated cotton fabrics, on cotton fabrics coated with wax only, and on cotton fabrics coated with a mixture of wax and mushroom fungus. It is expected that the wax coating will improve the water resistance and durability of the fabric, while the addition of mushroom mycelium may further improve the antibacterial, anti-inflammatory, and skin-friendly properties. This study also examined the potential environmental benefits of using mushroom mycelium as a natural additive that could reduce dependence on synthetic fibers and the use of chemicals for coating in textile manufacturing. The coating has advantages such as biodegradability and can improve the overall environmental footprint. Analytical techniques such as digital microscopy and Fourier-transform infrared spectroscopy (FTIR) were used to characterize the structure and composition of the coating. Mechanical tests were performed to evaluate the improved properties of the fabric. The results of this study indicate whether this coating is suitable for advanced textile applications and will contribute to the development of sustainable and high-performance fabrics, particularly in the textile sector.

#### 1.17. Monitoring the Impact of Global Climate Change by Analyzing Animal Groups in Thermophilic Fauna Corridors in Bulgaria

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Bulgaria is one of the smaller European countries, but it takes the leading place in faunal diversity, including cosmopolitan animal species and many Bulgarian and Balkan endemics. It is known that thermophilic and xerophilic Mediterranean animal species are found in the country's southern regions and along the Black Sea coast, and in northern mountainous areas, there are many species characteristic of Central and Northern Europe. No analysis exists for the percentage ratio between thermophilic and other faunal elements. This necessitates the examination of the routes for the penetration of thermophilic fauna. Some of the most significant potential corridors pass through the valleys of the rivers Struma, Mesta, and Maritsa, as well as along the Black Sea coast, and they are the main object of the research project we are presenting here. In 2022, we started to study these regions monthly—from their southernmost point on Bulgarian territory to the north, where Mediterranean influence is confirmed.

This project aimed to establish the species composition of various animal groups: arachnids (Arachnida: Araneae, Scorpiones, Solifugae), millipedes (Myriapoda), some insects (Insecta), terrestrial snails (Gastropoda), amphibians (Amphibia: Anura, Caudata), reptiles (Reptilia: Testudines, Squamata), and mammals (Mammalia: Rodentia, Eulipotyphla, Chiroptera), which are cosmopolitan, decisive for functioning ecosystems, and have proven bioindicator roles. During the research, indicator species were identified from the model groups, which can be used to monitor the long-term effects of global climate change. Combining different research approaches and various field methods for collecting material will contribute to achieving our set goal.

The overall completion of this survey will contribute to assessing the impact of anthropogenic pressure on individual species and species complexes. This can help develop strategies for managing the surveyed areas in a manner that is beneficial to humans and biodiversity.

#### 1.18. Diversity of Helminths in Snow Sheep (*Ovis nivicola*) and Muskox (*Ovibos moschatus*) in Russia

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Snow sheep (*Ovis nivicola*) and muskoxen (*Ovibos moschatus*) are Arctic ungulates. Snow sheep are endemic to Russia, while muskoxen were reintroduced from North America in 1974. Helminth fauna of these ungulates in the northern regions are being transformed under anthropogenic influence, and this requires additional research that considers helminth prevalence and pathogenicity.

More than 100 fecal samples of the snow sheep were collected during 2016–2024 in the Putorana Plateau (Krasnoyarsk Krai) and Republic of Sakha (Yakutia). More than 150 fecal samples of the muskoxen were collected during 2022 in the Yamalo-Nenets Autonomous Okrug, Wrangel Island, Chukotka Autonomous Okrug, Taymyr Peninsula (Krasnoyarsk Krai), and Zavyalov Island (Magadan Oblast).

Fecal samples were examined for the presence of macrohelminths (visible to the naked eye) and proceeded via larvoscopy, flotation, and sedimentation ovoscopy. The diagnoses were made based on the morphology and morphometrics of the obtained eggs and larvae.

The following helminths were detected in snow sheep: *Moniezia* sp.; small strongylids; *Marshallagia* sp.; *Nematodirus* sp.; *Protostrongylus* spp., (with a longer and a shorter tail spikes); *Trichuris* sp.; and *Capillaria* sp. The following helminths were discovered in muskoxen: *Moniezia* sp.; small strongylids; *Nematodirus* sp.; *Nematodirella* sp.; *Protostrongylidae* sp., (with a dorsal spine); and *Trichuris* sp.

*Capillaria* sp. was found in show sheep for the first time. Nematode larvae were sampled for DNA analyses. This research is to be continued.

### 1.19. Review of *Zygentoma* in Bulgaria with Report of Four New Species

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*Zygentoma* is a small ancient order of primitive hexapods with over 500 described species distributed worldwide. The original data about Bulgarian *Zygentoma* are scarce and refer to only four species: *Atelura montana* (Nicoletiidae), *Coletina bulgarica* (Nicoletiidae) (described from Bulgaria and known only from its first description), *Ctenolepisma lineatum* (Lepismatidae), and *Neoasterolepisma balcanicum* (Lepismatidae). In this study, the fauna of *Zygentoma* from Bulgaria was reviewed and extended using new distributional data with the addition of four more species: *Ctenolepisma calvum*, *C. ciliatum*, *C. longicaudatum*, and *Lepisma saccharinum*. The newly established species are from the Lepismatidae family and are synanthropic, showing increased spreading trends in recent years, except the *Ctenolepisma ciliatum*, which is not synanthropic but, in Bulgaria, has been found only in the vicinity of human dwellings. The collected specimens were preserved in 70-80% ethanol and deposited in the collection of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (IBER). This study is a part of the Project “Monitoring the effects of global climate changes through qualitative and quantitative analysis of model animal groups in selected corridors of thermophilic fauna penetration in Bulgaria” (funded by the National Science Fund of Bulgaria under Grant contract number KP-06-N61/6—14 December 2022).

### 1.20. Freshwater Ciliates in the Iberá Wetlands (Argentina): Ecological Importance and Diversity Patterns

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Ciliates are fundamental and diverse components of freshwater ecosystems. Despite their ecological importance, freshwater ciliates are understudied in Argentina, where research has primarily focused on taxonomic studies. Our investigation was conducted in Iberá (Corrientes, Argentina), one of the most significant subtropical wetlands, characterized by its vast extension and unique biodiversity. Between March 2022 and 2024, we sampled plankton from three Iberá portals: Carambola, Iberá, and San Antonio. Fixed samples were taken for quantitative analysis and live samples for qualitative analysis, along with physicochemical data. We assessed the composition of ciliates in terms of richness, analyzed  $\beta$ -diversity to compare ciliate compositions across different portals, and estimated multiple  $\beta$ -diversity. A total of 69 ciliate taxa new to Iberá were identified, distributed across ten groups, with Oligohymenophorea, Spirotrichea, and Litostomatea being particularly diverse classes in the studied region. San Antonio exhibited the highest species richness (55), followed by Iberá (27) and Concepción (14). This demonstrated notable variability in species richness between different sites, suggesting habitat heterogeneity. The  $\beta$ -diversity was high ( $\beta$ SOR = 65%), indicating that each portal has a unique and distinct species composition from the others. This indicated a high degree of differentiation among ciliate communities in the different Iberá portals. Multiple  $\beta$ -diversity analysis revealed that the main contributor to  $\beta$ -diversity was species turnover ( $\beta$ SIM > 50%). The high beta diversity and the predominance of species turnover highlight the importance of conserving

multiple sites within the Iberá region to preserve the total diversity of planktonic communities, such as ciliates. Each portal contributes a unique species composition, significantly contributing to regional biodiversity, thereby emphasizing the uniqueness of planktonic ciliate communities in the Iberá region.

### 1.21. Structural Role of Macrophytes in Testate Amoeba Communities in Aquatic Environments of the Iberá System

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The Iberá Natural Reserve (Corrientes, Argentina) shows off significant diversity in macrophytes, which, along with the chemical composition of its waters, profoundly influence the diversity of zooplankton communities. Testate amoebae play a crucial role in aquatic ecosystems. In Argentina, research on these organisms has been scarce thus far, requiring exhaustive studies on this group in the Iberá aquatic environments. For this study, sampling was conducted in June 2023 at Portal Carambola, where representative sites were selected, differentiating between vegetated and non-vegetated environments. Qualitative and quantitative samples were obtained, and in situ physicochemical variables were measured. Testate amoebae were observed in vivo using an optical microscope, and specific literature was followed for taxonomic classification. Richness (S),  $\beta$ -diversity, and multiple  $\beta$ -diversity were analyzed to compare the communities in the two environments and to determine the factors influencing them. The preliminary results demonstrated that testate amoebae communities in vegetated areas exhibited greater taxonomic richness (22 taxa) compared to communities in non-vegetated areas (8 taxa), with 6 taxa shared between the two. The  $\beta$ -diversity analysis, based on the Sørensen index (55%), suggested that the species composition of testate amoebae in both communities tends to differ between the two types of environments analyzed. The component that most contributes to the observed differences is  $\beta$ SNE (=30%). These results suggested that macrophytes provide resources and favorable environmental conditions that benefit a greater diversity of testate amoebae. Most of the exclusive taxa are found in vegetated areas, highlighting the specialization of some species in these resource-rich environments. The density and presence of macrophytes are key determinants for the diversity of testate amoebae communities. The reduction in macrophytes is associated with a decrease in taxonomic richness, underscoring their crucial role in these ecosystems.

### 1.22. Zooplanktonic Communities in a Lentic Environment of Northern Corrientes Province, Argentina

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**Introduction:** Zooplanktonic communities can be found in a wide variety of freshwater bodies. Often, the main groups represented are rotifers, cladocerans, copepods, protozoa, and fish larvae. Other groups of animals may also occur and are collectively referred to as tycho plankton or neuston. Records on planktonic communities in aquatic environments of Corrientes, Argentina, are scarce so far. The objective of this research was to study the zooplankton communities in a lentic environment of the northern Corrientes to elucidate their diversity in this ecosystem. **Methodology:** Sampling was carried out in April 2024 (autumn) at the Totorá Lagoon (27°22' S, 58°30' W), San Cosme (Corrientes, Argentina). Water samples were taken with plastic buckets from the littoral zone. Floating and submerged macrophyte plants were extracted. In situ measurements of physicochemical water parameters were recorded. In vivo samples were observed by examining macrophyte leaves and roots using a stereoscopic binocular loupe and a compound microscope.

**Results:** Oxygen levels were 7 mg/L, conductivity was 91  $\mu\text{S}/\text{cm}$ , and pH was 7.6. The water temperature reached 27.1  $^{\circ}\text{C}$ , with 46 ppm of dissolved solids. A total of 14 phyla and 46 genera were identified. The most diverse groups were ciliophora, rotifers, and amoebzoa. **Conclusions:** These results demonstrated the presence of a remarkable diversity of species in the Totorá Lagoon. Additionally, indicator species for specific environmental conditions were identified. This study presented the first record of planktonic communities in a lentic environment in Corrientes, Argentina, contributing to regional diversity. These findings could prove valuable for future research in the fields of ecology, biogeography, and biology.

#### 1.23. The Fauna Inventory of Rodents from the Echimyidae and Cricetidae Families in a Mining Area of Influence in the Amazon Region

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The rodent families Echimyidae and Cricetidae are among the most diverse groups of mammals in Neotropical forests. There are 267 species of rodents in Brazil, of which 72 are endemic to the Brazilian Amazon. These rodents play a crucial role in forest dynamics as sensitive indicators of habitat and landscape change, as well as displaying a wide range of karyotypic diversity, including diploid numbers (2n) from 10 to 118 and autosomal fundamental numbers (FNa) from 14 to 168. The current study focused on the chromosomal diversity of rodents from the Echimyidae and Cricetidae families from the hydro mining area in Paragominas, Pará, Brazil. Cytogenetic analysis was conducted using conventional staining, C-banding, and Fluorescence In Situ Hybridization (FISH) with telomeric probes on the species collected. We obtained the karyotypes of seven species: *Calomys tener* (2n = 66/Fna = 66), *Hylaeamys megacephalus* (2n = 54/Fna = 62), *Necomys lasiurus* (2n = 34/Fna = 34), *Oligoryzomys* sp. (2n = 62/Fna = 60), *Pseudoryzomys simplex* (2n = 56/Fna = 54) (Cricetidae), *Makalata didelphoides* (2n = 66/Fna = 84), and *Proechimys roberti* (2n = 30/Fna = 56) (Echimyidae). FISH using telomeric probes revealed only distal signals in all seven species' chromosomes. The karyotypes of all of the species were consistent with those previously described in the literature. We increased the distribution area of *Calomys tener* and *Pseudoryzomys simplex* to the Belém area of endemism, a species which can be typically found in the Cerrado region. This is evidence that the Amazon has become increasingly open due to deforestation and environmental degradation. The cytogenetic data from these species provide valuable insights for fauna mapping, conservation efforts, and evolutionary history.

#### 1.24. Parasitoid Communities in the Understory of a Forest in Poland Vary with Canopy Species Composition but Show Limited Responses to Herbivore-Induced Volatile Emissions of Oak Saplings

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Information on how parasitoids of herbivorous insects use herbivore-induced volatile organic compounds (VOCs) to find potential hosts on saplings in forests with varying tree composition is scarce. Therefore, we performed an experiment where we placed pairs of oak saplings (*Quercus robur* or *Q. petraea*) in neighborhoods dominated by oak, beech, or pine trees in a forest in Poland. We treated one sapling in each pair with the phytohormone methyl jasmonate, which triggers induced responses in plants. We measured the VOC emissions of thirty-six saplings and placed Malaise traps with five of the pairs. We counted the parasitoids in the ten Malaise samples and identified them using DNA metabarcoding. The two oak species differed in both the proportions of VOCs and the specific VOCs that were elevated following the application of methyl jasmonate. The parasitoid community appeared hyper diverse: our study included 1129 individuals belonging to 344 species, and most species could not be linked to a named species using DNA metabarcoding. We did not detect any overall effects of treatment on parasitoid abundance or community composition. However, some parasitoid species appeared to be attracted to elevated emissions of specific induced VOCs. Parasitoid communities differed significantly between areas and showed marginally significant differences between neighborhoods. Overall, our results suggest that parasitoids in the understory are affected by tree composition, although the effects of VOC emissions were limited. Nevertheless, our study demonstrated how VOC-mediated insect–plant interactions can be effectively investigated under field conditions.

#### 1.25. DNA Barcoding of Two Insectivorous Bats Species from Punjab, Pakistan

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Species identification based on mitochondrial gene cytochrome c oxidase subunit I (COI) is now considered as a powerful marker in molecular taxonomy. The current study was conducted with the aim to test the efficacy of the COI gene in randomly collected bat specimens from two different areas of Punjab, Pakistan. The carcass bat samples were collected randomly from two areas in Punjab, Pakistan (District Pakpattan and District Bahawalpur), and further processed for DNA extraction in a laboratory with a DNA extraction kit. The barcode gene was amplified using the primer pair PBCOIdF and PBCOIdR1 for bats collected from District Pakpattan and primer pair PBCOIdF and PBCOIdR2 for bats collected from the Bahawalpur District. The barcode sequences of specimens collected from the Pakpattan District (n = 4) and the Bahawalpur District (n = 3) revealed a 100% identity match with the species *Sotophilus heathii* and a 97–100% identity match with species *Taphozous nudiventris* on NCBI BLASTN, respectively. The sequences generated in the study were clustered through a neighbor-joining tree along with an additional dataset from GenBank of similar and closely related species from other countries. The intraspecific and interspecific genetic distances were also calculated, which showed a definite relation with the geographical distances of similar and closely related species from other locations. The sequences generated in the study were submitted to GenBank and can be used as a public database through assigned accession numbers.

This study strongly supports the credibility of DNA barcoding for species identification and encourages scientists in Pakistan to conduct more studies based on molecular taxonomy.

1.26. *Plasterer and Leaf-Cutter Bees (Hymenoptera: Colletidae, Megachilidae) of a Steppe Community on the Balkan Peninsula: Floral Hosts and Diversity in a Sub-Mediterranean Dry Grassland*

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The apoid fauna of Bulgaria is considered one of the richest in Europe. However, despite the numerous sampling efforts over the last century, it remains insufficiently studied, and a clear overview of its diversity and the ecology of species present is lacking. This lack of understanding is further exacerbated by the changing climate, which continually alters the northern and southern limits of species distributions, especially in the warm southern parts of the country. In this study, the megachilid and colletid fauna of a dry sub-Mediterranean steppe habitat were surveyed using standardized transect walks, where all bees belonging to the two families were collected, and their floral visits were recorded. Flower abundance was noted during each survey, and pollen was taken from the identified species for a reference collection. A total of 76 plant and 31 bee species were observed, with 45 individual plant–pollinator associations being identified at a species level. The most visited plants were the flowers of *Centaurea saloniitana* Vis. (six species), *Eryngium campestre* L. (four species), *Ajuga chia* Schreb. (four species), *Ornithogalum montanum* Cirillo (three species), *Onobrychis arenaria* (Kit.) DC. (three species), and *Knautia arvensis* (L.) Coult. (three species). Two species, *Hoplitis pici* (Friese, 1899) and *Megachile semicircularis* van der Zanden, 1996, are reported as new for Bulgaria, and twenty-two species are new for the locality. Considering the relative popularity and available historical data from the study location, the Besapari Hills, these results re-confirmed the apoid fauna of the country as severely understudied, further accentuating the need for a comprehensive pollinator monitoring in Southern Europe.

1.27. *The Diet and Niche Overlap of Two Cichlid Species in the Volta Grande Region of the Xingu River, Pará, Brazil*

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Trophic ecology is essential for understanding ecosystems and species interactions. Phylogenetically close groups can coexist in the same habitat and feed on similar items, resulting in niche overlap, which can compromise individual survival. In the Amazon, variations in river levels due to floods and droughts directly affect access to food resources for fish fauna. The objective of this study was to assess the influence of river discharge on the composition and dietary niche overlap of sympatric species *Geophagus argyrostictus* and *G. altifrons* in the Volta Grande of the Xingu River (Pará, Brazil). Monthly collections were conducted over a twelve-month period, and the specimens' diets were analyzed in a laboratory, with food items identified and weighed. The data were subjected to the Food Importance Index (IAi%) and Pianka's Overlap Index. The diet of *G. argyrostictus* consisted mainly of sediment (90.6%) and terrestrial plants (6.3%); *G. altifrons* were fed on sediment (60.7%), terrestrial plants (31.4%), and aquatic insects (6.5%). We observed variations in diet composition between dry and wet seasons (PERMANOVA;  $p = 0.008$ ) and between flood and recession periods ( $p = 0.037$ ) for *G. argyrostictus*, as well as variations between flood and dry seasons ( $p = 0.004$ ), flood and recession periods ( $p = 0.001$ ), and recession and dry seasons ( $p = 0.001$ ) for *G. altifrons*. Regarding niche overlap, Pianka's index values ranged from 0.39 during floods to 0.99 during dry seasons, with a significant



effect of river discharge on niche overlap (GLM;  $t = -2.234$ ,  $p = 0.049$ ), which was higher during low-water periods compared to high-water periods. Our findings highlighted the influence of river discharge on the diet composition of both species, which was more diverse during high-water periods. Also, we observed a greater dietary niche overlap during low-water periods, likely due to limited access to varied food resources, potentially resulting in interspecific competition.

#### 1.28. New Records for the Ground Beetle Fauna of Bulgaria (Coleoptera: Carabidae)

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Bulgarian ground beetles (Coleoptera: Carabidae) have been studied intensively by many native and foreign scientists. Here, we presented three new species for the country. The data were obtained between 2001 and 2021 on several field trips.

*Agonum carbonarium* Dejean, 1828 is a hygrophilous beetle with very disjunct populations dwelling in bog-like habitats in high-mountain belts. It inhabits a vast area ranging from the Pyrenees over the mountains of Europe and the Caucasus to the Far East of Russia. It is a polytypic species with four subspecies. Populations of *A. carbonarium* from the Balkan Peninsula still have an unclear taxonomic status, mostly due to the lack of material. This species has wide pronotum and fovea-like setiferous pores on the third elytral interval, and it cannot be mistaken with any other *Agonum* known in Bulgaria.

*Harpalus fuscipalpis* Sturm, 1818 has a wide distribution, from central Europe to China, and is also found in the Nearctic region. It has been considered a synonym of *Harpalus fuscicornis* Sturm, 1818, but there are proven differences in their morphology and aedeagus, confirming their separation.

*Lionychus fleischeri* Reitter, 1908 has a limited distribution in just a few territories in Europe, where it is represented by three subspecies. The discovery of *L. fleischeri* in Bulgaria raises questions about its taxonomical belonging. It most likely belongs to the ssp. *fleischeri*, but it is possible that it actually represents a separate form. The species differs from *Lionychus quadrillum* (which is widely distributed in Bulgaria and Europe) by the absence of spots on the elytra—they are unicoloured, black, glossy, with a green metallic sheen by the hind angles of the pronotum, which are without distinct denticles.

The first species was collected from 2450 m a.s.l. in the Rila Mts., while the other two species were collected from the Struma River valley.

#### 1.29. Notes on Genus *Streblocera* Westwood (Hymenoptera: Braconidae) from India with First Report of Subgenus *Asiastreblocera*

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Euphorinae is one of the largest subfamilies of Braconidae with more than 1270 described species worldwide [5]. In India, as of now, a total of more than 60 species were described under 15 different genera. The genus *Streblocera* Westwood is one of the unique genera among the euphorines, with female specimens having raptorial-like antennae and long scapes. Members of this genus are known to be koinobiont endoparasitoids of adult chrysomelid beetles with more than 120 valid species present worldwide under five subgenera: *Asiastreblocera*, *Cosmophoridia*, *Eutanycerus*, *Villocera*, and *Streblocera* [6]. *Streblocera* is still an understudied group in many parts of India with only ten species recorded under

the subgenus *Eutanycerus* until now. Among them, nine species were recorded from Uttar Pradesh and Madhya Pradesh States [7] and one species was reported from Tamil Nadu State [8]. The subgenus *Asiastreblocera* can be differentiated from other subgenera by having a face with an acute horn and fifth metasomal sternite with a pair of acute teeth [9]. A total of six species under the subgenus *Asiastreblocera* are reported from the Oriental region (China, Korea, Taiwan, and Thailand). In the present study, we reported the occurrence of the subgenus *Asiastreblocera* for the first time in India, along with a list of known species in India.

1.30. *Stealing a Spot Among the Oriental Myrmecofauna—The First Record of Invasion of Mainland Eurasia by the New World Thief Ant Solenopsis Globularia*

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The genus *Solenopsis* has attained notoriety around the globe for housing the aptly-named fire ants; *S. geminata* (Fabricius) and *S. invicta* Buren, in particular, have made their presence painfully known far outside their native ranges in the New World. Lesser in both stature and renown, their congeners, the thief ants, however, have yet to exhibit the invasive potential of their larger relatives or have achieved so while evading collection (due to their cryptic behaviour) or subsequent identification (due to the difficulty of their taxonomy). The findings presented here support the latter hypothesis, with a member of the thief ants being recorded for the first time from mainland Eurasia. *Solenopsis globularia* (Smith) is among the larger of the thief ants, with a similarly broad distribution in the New World. It was also recorded as an exotic species in Africa and on islands in the Atlantic and Pacific, including the Philippines, which, until now, has represented the only part of its range that extended into Eurasia. Here, documented for the first time, is the presence of *S. globularia* in India, which was previously known to harbor only two *Solenopsis* species, *S. nitens* Bingham and the invasive *S. geminata*, from which it can be easily distinguished by its greatly dilated, globose postpetiole. All specimens were collected opportunistically from an urban area, with the simultaneous recording of associations with other taxa. The absence of detailed ecological observations of this species, even in its natural habitat, make it difficult to predict the consequences of its introduction into the Old World, and further research is needed to determine the level of threat posed to native taxa.

1.31. *Diversity and Spatial Distribution of Benthic Crustaceans in Southern Tunisian Coasts*

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Crustaceans represent an important group of macrofauna benthic in the coastal marine ecosystems, constitute important linkages in the food web, and can respond nonlinearly to environmental changes. Crustaceans' diversity of the Gulf of Gabès was studied in eight localities in southern Tunisian coasts during the last years (2019–2024), and Benthic crustaceans sampling in the southern Tunisian coasts was carried out using a Van Veen grab, covering an area of about 0.05 m<sup>2</sup>. In total, 20,114 individuals belonging to 262 crustacean species were identified. Amphipoda (48% total species), Decapoda (23%), and Isopoda (16%) are the most dominant taxa. Seagrass meadows (*Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltei*) constitute a suitable habitat for crustacean species in the Gulf of Gabès and include high diversity. The multivariate analysis (MDS, ANOSIM, . . .) revealed significant differences in the composition and trophic diversity of crustaceans' assemblages among the eight studied sites and showed that the crustaceans' distribution in the southern Tunisian

coasts correlated with many natural and anthropogenic factors, such as depth, sediment type, organic matter, and pollution gradient. This study suggested initiating a long-term monitoring program to improve our understanding of the temporal changes in crustacean communities in the Gulf of Gabès to recommend the necessary conservation measures in this area of high-value natural heritage.

### 1.32. Chalcid Wasps (Hymenoptera: Chalcidoidea) Associated with Plant Galls in India

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Chalcidoidea is the second largest superfamily after Ichneumonoidea, and they have a greater range of biological diversity than any other parasitic superfamily. They are mostly parasitoids but are also known to be phytophagous. Chalcids associated with plant galls can induce galls or occur as parasitoids of gall-forming insects and, in some cases, exist as inquilines. A preliminary study was carried out in Karnataka, India, during the years 2023–2024 to document the association between chalcid wasps and plant galls. During surveys, leaf galls were collected from *Acacia chundra* Rottler, *Carissa spinarum* L., *Erythrina* sp., *Ficus racemosa* L., *Pongamia pinnata* (L.), *Terminalia arjuna* (Roxb.), and two other undetermined plants. The galls were placed inside rearing containers and monitored for wasp emergence. It was observed that among the galls sampled, the gall niche was mostly utilized by members of the family Eulophidae (Subfamily: Tetrastichinae). In the present study, a pteromalid wasp—*Cheiropachus* sp.—was newly recorded from leaf galls of *Carissa spinarum*, which presents an interesting observation since members of genus *Cheiropachus* was previously only recorded from Coleoptera (Bostrichidae, Cerambycidae, Curculionidae, and Scolytidae) hosts. A checklist of chalcid wasps and their associated plant galls was provided and their likely hosts.

### 1.33. Investigation of the Number and Types of Venation Anomalies in Honey Bee Fore Wings

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**Introduction:** Wing venation is used in the taxonomy of insects such as ants [10], honey bees [11,12] and others. Accounting for venation anomalies can be successfully achieved in population monitoring [13]. Many authors studied natural venation anomalies in honey bees. Their various types and localizations were described [11,12,14].

**Methods:** The work on accounting for anomalies in the venation of the fore wings was performed in the laboratory of the FSBSI “FBRC” by viewing images containing the fore wings (left and right) of honey bees and registering these anomalies in a specially designed form.

**Results:** It was established from literary sources that there are 22 types of anomalies in the venation of the front wings of honey bees. By analyzing the obtained wing images, we identified 17 types of anomalies, of which 6 are not described in the literature. The anomalies encountered in the analysis of wing images were recorded in the form we developed. Photos of the found anomalies were taken. A total of 2240 pairs of front wings were analyzed, of which 794 pairs had at least one anomaly. The most common types of anomalies were “c” and “j”. The average number of anomalies per bee was  $0.41 \pm 0.034$ , with fluctuations from 0.05 to 1.32 and a coefficient of variation of 61.4%.

**Conclusions:** The frequencies of the occurrence of different types of wing venation anomalies were established, of which type “j”—57.6% and type “c”—15.9% were the most common. The average number of anomalies per bee was  $0.41 \pm 0.034$ . In total, and six types of anomalies not described in the literature were determined.

### 1.34. Hotspots of Endemism: European Salamanders and Newts

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This study explored endemism among salamanders and newts (order Caudata) in Europe, emphasizing their critical role in biodiversity conservation. Endemism, denoting species unique to specific geographic locations, makes these species particularly susceptible to extinction from habitat loss, climate change, and other environmental pressures. European Caudata exhibited notable endemism due to diverse habitats and historical events like glaciation, creating isolated environments conducive to the evolution of endemic species. Data collection utilized the IUCN Red List to compile a comprehensive list of 40 Caudata species in Europe, identifying 27 as endemic. Detailed distribution areas were obtained as ArcGIS shapefiles from the IUCN Red List, facilitating the identification of endemism hotspots via Geographic Information System (GIS) analysis. This method enabled the precise mapping and analysis of habitat ranges, ensuring targeted conservation efforts. The study identified key regions in Europe with high levels of Caudata endemism, including Corsica, Sardinia, the Iberian Peninsula, Northern and Southern Italy, Greece, Central Europe, the Carpathian Mountains, the Pyrenees, France, the Southwestern Alps, and the Dinaric Alps. These regions are characterized by unique environmental conditions that promote the development and persistence of endemic species. The study concludes that European Caudata's high endemism is driven by diverse habitats, geographic isolation, varied climatic conditions, and historical factors. The findings underscore the importance of protecting these unique environments to preserve Europe's rich biodiversity, highlighting the necessity for ongoing research and conservation efforts to protect these vulnerable species from environmental changes and human activities.

### 1.35. Sulfidic Caves in South-East Albania—A New Hotspot of Subterranean Biodiversity

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Caves represent aphotic subterranean environments characterised by the absence of photosynthetic primary production. The discovery of a sulfidic subterranean ecosystem in Movile Cave (Romania), in 1986, followed by similar discoveries made later in other sulfidic caves, showed that significant trophic resources can be produced chemoautotrophically in situ underground. These resources can support exceptionally abundant and diverse subterranean biological communities, including numerous endemic invertebrate species. To date, every sulfidic cave ecosystem explored has emerged as a hotspot of subterranean biodiversity.

Here, we report on two sulfidic hypogenic cave systems recently explored in south-east Albania in the Vromoner and Langarica canyons. These caves were formed through sulfuric acid speleogenesis, as evidenced by the large subterranean deposits of gypsum and sulfur that cover the cave walls. Recent field observations and surveys revealed abundant sulfur-oxidizing biofilms and rich and diverse invertebrate communities inhabiting these caves. Light carbon and nitrogen stable isotope ratios support the hypothesis that subterranean foodwebs are chemoautotrophy-based, relying on in situ carbon fixation. To date, ten endemic arthropod species have been identified here (scorpions, pseudoscorpions, spiders,

springtails, centipedes, and beetles). Notably, a massive colonial spiderweb, hosting over 50,000 specimens of *Tegenaria domestica* (Agelenidae), was found covering the limestone wall in a sulfur cave. Several species of bats have been identified, including a rare species (*Myotis bechsteinii*), and large maternity colonies were discovered and surveyed. Our primary focus is on the subterranean biodiversity, ecology, and geomicrobiology of these caves.

We are engaging with local stakeholders to foster conservation actions for the protection of these unique sulfidic caves and for the conservation of the biological communities they host. In collaboration with European initiatives “Save the Balkan Rivers” and “The Blue heart of Europe”, we advocate for the inclusion of these canyons and their caves in the regional national parks of Greece and Albania.

### 1.36. Diversity and Conservation Status of Amphibians and Reptiles in Rudnik Mountain (Central Serbia)

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The Rudnik mountain region in central Serbia is rich in many habitats, including forests, meadows, pastures, rocky surfaces, and numerous springs and aquatic ecosystems. All of these environments are suitable for the majority of animal species, including amphibians and reptiles. However, there has been insufficient research on the amphibian and reptile species in this region. We conducted a field study on Rudnik Mountain in 2021 to compile a comprehensive inventory of amphibian and reptile species. We determined the presence of species by first-hand observations of adult or larval forms, identifying distinctive markings, and detecting groups of deposited eggs or larvae. A total of 8 species of amphibians and 12 species of reptiles were recorded. The data gathered during this research are preliminary, although they make a substantial contribution to the understanding of the fauna in this region. According to national legislation, three of the recorded species are without any protection, three of them are classified as protected, and the other species are classified as strictly protected. Various factors, principally caused by human activities, contribute to the decline in the number of these species populations. Undoubtedly, there are methods available to safeguard the populations of various animal groupings, such as promoting education, conducting further scientific research, implementing long-term monitoring, and restoring certain habitats.

### 1.37. Multi-Scale Comparison of the Shapes and Drivers of Stream Fish Species–Area Relationships in a Regional Aquatic Biodiversity Hotspot

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The species–area relationship (SAR), which predicts species richness scales positively with increasing area, is among the longest-recognized trends in ecology. Despite its ubiquity, our knowledge of the precise shapes and underlying mechanisms driving SARs remains incomplete, particularly in freshwater ecosystems. I tested theoretical predictions for SAR shapes and best-fit models and evaluated SAR scale dependency across the nested sampling scales of sites (finer scale) and hydrological units (HUs; intermediate scale), while using stream fish communities from the Alabama aquatic biodiversity ‘hotspot’ as my model system. Using data from 599 standardized fish surveys, I conducted model selection and multimodel averaging on 20 SAR models (e.g., logarithmic, power, logistic) and I examined the influence of three main hypotheses influencing SARs—passive sampling

effects, disproportionate effects, and heterogeneity. I also used multiple linear regressions to test environmental drivers of SARs. Despite model uncertainty, my results supported scale dependency of stream fish SARs and their best-fit models; however, more flexible nonlinear models generally outperformed other models, and the Monod (i.e., Michaelis–Menten) model provided a plausible fit across scales. Despite partial sampling effects, the null “passive sampling hypothesis” was rejected in favor of disproportionate effects of increasing area, mediated most prominently through additions of common species, rather than rarer species. Multiple regressions revealed that a greater percentage of variation in freshwater fish species diversity at the sampled scales is explained by area than latitude or other environmental factors. Understanding the mechanisms driving SAR results has important implications for conservation and management, including aiding reserve design as well as development of predictions for biodiversity change in response to habitat loss or fragmentation.

### 1.38. Notes on Chalcidid Wasp (Hymenoptera: Chalcididae) Fauna from Central India

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Chalcididae is one of the important families in Chalcidoidea in terms of biological control. Its members are pupal parasitoids of most lepidopterans and can be easily differentiated by having an enlarged hind femur with variable teeth on the ventral side. Currently, more than 1500 valid species across five subfamilies have been documented worldwide. In India, the Chalcididae fauna was extensively studied during the 1990's, and more than 250 valid species were found to be present under five subfamilies, while only 14 species are reported to be from central India. In this study, surveys were undertaken from 2019 to 2023 to document the chalcidid wasp fauna from central India, with special reference being made to Chhattisgarh state. Random surveys were taken in more than 20 sites across the Chhattisgarh state, including the permitted forest areas, and an abundance of chalcidid wasps situated at various altitudes across four elevation gradients was recorded. A total of 350 individuals belonging to four subfamilies were collected, and the documentation of more than 50 species across eleven genera was performed. Haltichellinae and Dirhininae were the most dominant subfamilies, while Epitraninae was the least documented one in terms of abundance, and *Brachymeria*, *Antrocephalus*, and *Hockeria* were found to be the most speciose genera. Based on the surveys, Chalcididae fauna are more diverse in 250–500 msl and 750 msl gradients. *Dirhinus himalayanus* and *Dirhinus claviger* have been found across the gradients, while many of the species were restricted to particular elevation ranges. Species diversity is discussed with respect to elevation gradients, and an irregular spread has been found across the state.

### 1.39. The Curation and Organization of Samples for Molecular Studies in the Fish Collection of Museu de História Natural da Bahia, Bahia, Brazil

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The ichthyological collection at the Museu de História Natural da Bahia (MHNBA) in Bahia, Brazil, started in the 1970s, initially focusing on marine species. Between 2003 and 2024, several field expeditions were conducted to explore the taxonomic and geographic diversity of freshwater species across eight Brazilian ecoregions. Since 2005, and especially in the last seven years, expeditions funded by Brazilian development agencies have been carried out to collect fish tissues, establishing the fish tissue collection at MHNBA. In 2019, the collection began to be digitized, and a standardized sample management procedure was implemented. Currently, the ichthyological collection at MHNBA, with material preserved

in formalin, consists of approximately 10,500 catalogued lots from about 1600 locations, stored in 70% alcohol and housed in sliding cabinets. The tissue collection is stored in vertical freezers at  $-18^{\circ}\text{C}$ , organized in Eppendorf tubes (aliquots and whole specimens), and distributed in numbered boxes and drawers. All information is recorded in the cataloguing system to facilitate access and curation. To date, 6044 specimens from 105 genera and 263 species have been catalogued. This collection also includes samples of 29 paratypes from four freshwater fish species. The number of species is likely underestimated, as individuals identified only to the generic level were excluded from the count, and many lots still need to be catalogued. Additionally, the collection includes samples of species that are uncommon in tissue collections, such as *Hasemania piatan*, *Lignobrycon myersi*, *Moenkhausia diamantina*, and *Hyphessobrycon negodagua*. The fish tissue collection at MHNBA is one of the most significant in the Brazilian Northeast and has been instrumental in developing various projects, primarily focused on the integrative taxonomy and phylogeography of freshwater fishes.

#### 1.40. Aquatic Invertebrate Assemblages as Proxies for Adjacent Karst Spring Differentiation

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Karst springs are extremely diverse in terms of morphology, microhabitat structure, hydrology and hydrogeology and consequently unique in their composition of aquatic invertebrate communities. The two studied karst springs of the Gacka River in Croatia, Majerovo and Tonkovića Vrilo, are large limnocrenous springs located in different sub-catchments. They differ in terms of groundwater residence time, discharge regime and self-purification capacity of the water. Invertebrate meiofauna and macrozoobenthos samples were collected seasonally following the modified AQEM protocol to (1) assess the structure and abundance of the benthic assemblages in different microhabitats in relation to ecological and spatial correlations in each spring; (2) assess and compare the habitat preferences of the aquatic invertebrate community; and (3) assess the variation in habitat preferences at the microhabitat level. A total of 70 invertebrate subsamples from dominant inorganic and organic substrates were collected along 100 m of each spring. Most physico-chemical water parameters differed significantly between the two springs, with values generally lower in Majerovo Vrilo. Primer 6.1 SIMPER analysis identified vegetation and animal taxa that were most responsible for the multivariate distance between seasonal samples and showed that isopods and amphipods were most abundant in both springs during all sampling periods. Invertebrate species richness was significantly higher in Tonkovića Vrilo, where the higher concentrations of dissolved ions were measured. Non-metric multidimensional scaling (nMDS) of ordination plot revealed a separation of invertebrate assemblages between springs at the microhabitat level. The classification of vegetation-animal assemblages into types based on microhabitats is intended to characterize the life requirements of each recorded species. This study has shown the importance of overlaying



multifunctional ecological data to assess land-use changes in different sub-catchments of two neighbouring springs.

#### 1.41. Genomic Approaches to Reveal the Genetic Components of the Peruvian Paso Horse

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The Peruvian Paso Horse (PPH) is a breed native to Peru. It is important socially and economically and is classified as a flagship product. However, very little is known about its genetics, thus limiting the development of a genetic improvement program and conservation. Even though genomic tools are widely available for different animal species of importance, such as horses and cattle, the PPH remains abandoned. Moreover, its phylogenetic relationship, together with the genetic and population structure components of the PPH, are still limited. Here, we sequenced the whole genome of a representative individual of the PPH using long and short reads and obtained 160 GB and 74 GB of raw data, respectively. Hi-C sequencing will show the three-dimensional chromosome structure of this breed, and RNA-Seq reads will determine its gene expression. In addition, a total of 150 hair samples from the tail and mane of the PPH were recently collected in the geographic departments of Lima and Arequipa in order to genotype them with an SNP chip, attempting to determine the genetic structure and diversity of this Peruvian breed. Currently, we are in the process of sending the DNA samples to an external service. In the meantime, we are using different bioinformatic tools to assemble and annotate the mitochondrial and whole genome of the PPH. We expect to obtain our final results before the year comes to an end. Finally, the use of these modern tools may stimulate other Peruvian researchers to study our abundant zoogenetic resources for conservation and breeding purposes, promoting their sustainable use.

## 2. Biodiversity Conservation

### 2.1. Wildfire Influence on the Assemblage of Bees and Wasps (Hymenoptera) in El Impenetrable National Park

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**Introduction:** Wildfires, mostly caused by human activities, represent a growing threat in Argentina and are aggravated by climate change, which increases favorable conditions for their spread. In addition to their devastating effects on vegetation, they generate smoke emissions that negatively affect human health and fauna, especially insects. This study focused on El Impenetrable National Park, where bee and wasp populations were compared before and after the fires that occurred in 2022. **Methods:** Traps were set in different areas of the park to capture these insects in November 2021 (before the fires) and November 2023 (after the fires). **Results:** Seventeen species/morphospecies of bees and wasps were identified, and a decrease in species richness was evidenced after the fires. Beta diversity revealed a particular species composition each year, with a significant loss of species after the fires. This decrease in richness and diversity can be attributed not only to the direct effects of the fires but also to the effect of smoke on the behavior of flying insects. **Conclusions:** Wildfires negatively affected the assembly of bees and wasps in El Impenetrable National Park in 2022, initiating a post-fire period with low levels of richness and diversity. The importance of monitoring these insects and studying the fire regime of the region is emphasized to better understand the complex interactions in ecosystems and propose actions for the conservation and sustainable usage of the resources.

## 2.2. Revolution in Conserving and Detecting Biodiversity: Environmental DNA (eDNA) Technique

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Nature's unique mechanisms hold the key to some promising strategies. In particular, nature's self-cleaning mechanism against pollution burdens both protects ecosystems and prevents the degradation of biodiversity. Although Environmental DNA (eDNA) is a new concept today, it represents DNA obtained from any environmental sample. That is, it includes DNA collected from different ecosystems without an isolated organism, originating from body cells or organism waste. eDNA studies have gained popularity in the last decade. This popularity is due to the fact that the eDNA technique never harms any ecosystem or biodiversity (including endemic species in water and soil ecosystems). Its environmental friendliness makes eDNA an efficient biomonitoring technique compared to traditional methods. Traditional monitoring methods include the visual identification or manual collection of organisms. The main disadvantages of these methods compared to eDNA are cost and time. eDNA is a revolutionary method for studying biodiversity in water and soil ecosystems. It is also used by environmental scientists to monitor living bio-indicators in cases of pollution in receiving water and soil environments. This review aims to review the literature on 'Environmental DNA (eDNA)' from different international scientific databases and to discuss the applicability and effectiveness of eDNA in the field of biodiversity.

## 2.3. Climate Change Impacts the Distribution of *Kelussia odoratissima* Mozaff., an Endangered, Monotypic, Endemic Plant in Chaharmahal and Bakhtiari Province, Iran

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**Introduction:** Endangered endemic medicinal plants are facing the risk of habitat destruction, overexploitation, and climate change. Predicting their potential distribution accurately using species distribution models (SDMs) can help prevent the loss of plant diversity and ecological destruction caused by climate change. *Kelussia odoratissima* Mozaff., also known as mountain celery, is a valuable edible and medicinal plant species that is unique to the central Zagros highlands. It is utilized in traditional medicine and contains bioactive components with pharmacological properties. However, due to the environmental impact of population growth, the plant is now at serious risk of extinction.

**Methods:** In this study, we utilized a maximum entropy model (MaxEnt) to determine the potential distribution of *K. odoratissima* in one of the main centers of its distribution, Chaharmahal and Bakhtiari province, under two representative concentration pathways (RCP 2.6 and RCP 8.5) for the 2050s and 2070s.

**Results:** The MaxEnt model was effective, as evidenced by an AUC value exceeding 0.9. The results indicated that the distribution of this taxon was primarily influenced by the soil organic carbon content, precipitation seasonality (Bio15), and precipitation of the wettest month (Bio13). The model predicted a reduction in suitable habitats for *K. odoratissima* under all scenarios in the 2050s and 2070s.

**Conclusions:** These findings are significant for the preservation, management, and continued study of this endangered and medically valuable plant in Chaharmahal and Bakhtiari province.

#### 2.4. New Records of Sea Anemones (Cnidaria, Anthozoa, Actiniaria) from Namuncurá-Burdwood Bank Marine Protected Area I, Area II and Adjacent Areas

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The Namuncurá-Burdwood Bank Marine Protected Area (N-BB MPA) is the first of its kind located on the Continental Shelf of the Argentine Republic. It was designated as an MPA in 2013 due to its oceanographic characteristics, the significance of its ecosystems with a high degree of biodiversity and endemism, and the potential for human impact. Geomorphologically, the Burdwood Bank (53°40'–55° S; 62°–60°40' W) consists of a submerged plateau with an approximate area of 28,000 km<sup>2</sup>, with depths ranging from 50 to 200 m on the plateau's surface and extending to 4000 m on the southern bounding slope. In 2018, the southern slope was incorporated as a conservation area, resulting in the formation of the Namuncurá-Burdwood Bank II MPA.

Sea anemones represent a significant component of intertidal and subtidal benthic communities, influencing their structure and function. Understanding the biodiversity of sea anemones within the N-BB MPA is crucial, particularly for conservation decision-making. Preliminary reports and species lists of sea anemones within N-BB MPA I and II and adjacent areas have identified eight species: *Dactylanthus antarcticus*, *Bolocera kerguelensis*, *Isosicyonis alba*, *Actinostola crassicornis*, *Phelliactis* cf. *lophohelia*, *Hormathia pectinata*, *Actinauge langicornis*, and *Paractis leavis*. Detailed analysis of samples and data obtained from campaigns conducted in the area by CONICET (Argentina) in 2016 and 2017 has expanded this knowledge. This study adds the species *Stomphia selaginella* and *Scytophorus striatus* to the list of species present in the N-BB MPA and adjacent areas. Additionally, these are the first records of *S. striatus* on the Argentine Continental Shelf, thus expanding the list of sea anemones documented in Argentine territory.

#### 2.5. Ecological Role of *Polybia ruficeps* (Hymenoptera: Polistinae) in 'El Impenetrable' National Park, Chaco, Argentina

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**Introduction:** *Polybia ruficeps* is a eusocial wasp from South America, commonly known as the Red-Headed Camoati. These insects are quite docile and are characterized by collecting and storing sugary substances such as nectar, extra-floral nectar, or excretions from aphids for honey production. Also, they play a role as pollinators of native flora. **Methods:** In this study, the digestive tract of these wasps was examined to determine their foraging behavior (endo-entomopalynology) in a national park in northern Argentina. Specimens were collected from 'El Impenetrable' National Park (Chaco, Argentina) in November 2021. The gut contents were analyzed using safranin staining for observation under an optical microscope. Pollen grains and spores were subsequently identified. **Results:** Preliminary results show that in November, these wasps fed on Quebracho trees (Anacardiaceae) and Palm trees (Arecaceae), which are characteristic plants of the phytogeographic region. A large number of ascospores, didymospores, and phragmospores from fungi belonging to the Agaricaceae, Chaetomiaceae, and Pleosporaceae families were also observed. **Conclusions:** The same pattern was observed in other regions of Latin America concerning this wasp and the typical vegetation of the areas they inhabit. The fact that they feed on both trees and fungi that only inhabit the soil or dead wood suggests a possible preference for foraging these resources. Thus, they can play a role as dispersers of pollen and spores, which is important for the recovery of the forests that they inhabit.

## 2.6. Color Preferences of Flower Visitors in 'El Impenetrable' National Park, Chaco, Argentina

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**Introduction:** Flower color is an essential characteristic for attracting pollinators, as many of them exhibit innate preferences for certain colors. These preferences vary according to geographical environments, where the most commonly highlighted colors include violet, yellow, white, blue, and orange. This research aims to address the following question: what are the color preferences of different floral visitors in one of Argentina's most important national parks? **Methods:** Various orders of insects were collected using 80 pan traps of blue, white, red, and yellow colors. The samples were transported to the laboratory for identification and classification. **Results:** Nine orders of insects were identified, with Hymenoptera and Diptera being the most abundant. The colors yellow and white were found to be the most attractive for different groups of insects. Specifically, yellow primarily attracted bees and dipterans, while white was more attractive to bees and wasps. On the other hand, red and blue were not effective in attracting insects in the study area. **Conclusions:** The different groups of insects showed clear preferences for certain colors of the traps, reflecting their behavior in relation to the flowers they visit. This study used colored traps for the first time in this National Park and demonstrated that they are an effective sampling method for recording the color preferences of flower visitors. These contributions help us to understand plant–pollinator interactions in this ecosystem, providing information that can be used for conservation strategies and habitat management.

## 2.7. Crossing the Border of a Small Felid Conservation: A Case Study to Investigate the Threats to the European Wildcat in Hungary and Pallas's Cat in Mongolia

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The European wildcat *Felis silvestris* and Pallas's cat *Otocolobus manul* are listed as *Least concern* on the IUCN Red List. However, both cats face the same threats due to anthropogenic activity worldwide, and another threat is a lack of ecological knowledge. In this study, we investigated threats to both cats through interviews and an online questionnaire. We gathered data on wildcats from 206 respondents through Game Management Units in Hungary, and 107 herders were interviewed on a Pallas's cat survey in Mongolia in 2022. The results showed that the primary threat to wildcats was hybridization with domestic cats (*Felis catus*) 32%, followed by a lack of habitat 31%, and disease 12%. Roadkill and predators accounted for 6%, and poisoning and hunting 4% were other concerns. Meanwhile, lack of prey (13%) and lack of habitat (11%) were major threats to Pallas's cats in addition to herding dogs (7%). Nonetheless, 65% of respondents believed that there was no threat to Pallas's cats in the area. Our study suggests that a lack of habitat is a main threat for wildcats and Pallas's cats, and hybridization is thought to be a critical threat to wildcats, whereas Pallas's cats face a lack of prey. A robust understanding of small Felid's threats is paramount to defining sound conservation plans for future management in Hungary and Mongolia.

## 2.8. Survey on Future Distribution of South-Western Alps' Endemic Species *Gentiana ligustica* R.Vilm. and *Chopinet*

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*Gentiana ligustica* is an endemic species of the south-western Alps, protected by European laws, with populations growing under both alpine and mediterranean climates.

Their Conservation Status is assessed as “Good”, with a stable trend for alpine populations; meanwhile, for the Mediterranean populations, their Conservation Status is assessed as “Inadequate”, with an unknown trend due to insufficient studies and a habitat degradation that is still in progress and that can bring about the disappearance of the population to the point of species extinction.

To evaluate the impact of climate change on the species, we projected the potential distributions of *G. ligustica* under two different RCP scenarios (SSP3-7.0 and SSP5-8.5) for the years 2070–2100 by using two different sets of variables downloaded from the CHELSA database. The first set had five bioclimatic variables, which are considered physiologically important for the species even if they are correlated with each other, and the second set had three uncorrelated variables. Models were run in the R package “biomod2” by using 12 algorithms.

Under both scenarios and by using both sets of bioclimatic variables, the models suggest that the southernmost populations growing under a Mediterranean climate will be strongly negatively affected by climate change, facing extinction due to the loss of habitat suitability. Moreover, habitat suitability will strongly decrease in the alpine region, shifting in the northern direction toward high-elevation areas, resulting in a high extinction risk for a large part of the alpine populations as well.

This study underlines the need for proactive conservation plans and assisted colonization strategies in suitable areas to preserve *Gentiana ligustica* in the face of climate change.

#### 2.9. Effect of Forest Management on the Population of *Jacaranda copaia* (Aubl.) D. Don in the Brazilian Amazon

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The Brazilian Amazon faces environmental degradation due to inadequate forest management practices. This study evaluated the post-logging influence on the structure and dynamics of the species *Jacaranda copaia* in degraded Amazonian forest, aiming to improve sustainable forest management. Horizontal structure, mortality and recruitment dynamics, annual periodic increment in diameter, and diameter distribution were assessed in two blocks within the same area, over different monitoring intervals. Data were obtained from 50 permanent plots, each with 25 subplots of 10 × 10 m of continuous forest inventory (12.5 ha). This included 20 control plots (5.0 ha) and 30 logging plots (7.5 ha) for all trees with DBH ≥ 5 cm. Measurements were taken in 2009, 2012, 2014, and 2020 for Block I, and in 2011, 2015, and 2020 for Block II. The *J. copaia* population showed variations in the structure and dynamics over the years, influenced by logging. In Block I, the population increased by approximately 16.00% between 2014 and 2020, following the 2013 harvest; the basal area also increased 0.311 m<sup>2</sup> ha<sup>-1</sup> or 76.41%. In Block II, after logging, the population decreased approximately 11.11% between 2015 and 2020, while the basal area increased 0.221 m<sup>2</sup> ha<sup>-1</sup> or 33.18%. The recruitment rate exceeded the mortality rate throughout the study period, indicating a continuous process of regeneration and population growth of *J. copaia*. Diameter growth in Block I was 0.72 cm year<sup>-1</sup> over a period of 11.2 years, while in Block II, growth was 0.58 cm year<sup>-1</sup> over a period of 9 years. Thus, forest management benefited the *J. copaia* population, with a positive impact on the population structure and dynamics, demonstrating its post-logging recovery capacity.

### 2.10. Is Wild Boar Rooting a Form of Damage or an Effect? Combined Impacts of Wild Boar Rooting and Water Erosion on Some Soil Properties

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Wild boar activities are well known and well described worldwide. They cause a lot of conflicts, but their measured parameters are rarely published, with the literature concentrating on the emotional perceptions of the matter. Furthermore, as we go into detail, e.g., the wild boar and pedology or the wild boar and erosion, we find even less research on these connections/topics. In a recent research study, we examined a Hungarian grassland with 50% bush cover on a steep slope that could result in considerable soil water erosion. The null hypothesis was that the wild boar causes differences in soil properties at different scales in the eroded and sedimented parts of slope sections. Soil samples from wild boar rootings (20–50 cm deep), from the ring around these rootings (15–25 cm high) and from nearby control areas (1–2 m distance from the ring) were collected from the upper and the lower third sections of the slope. Samples were analyzed with a near-infrared soil scanner (Agrocares Ltd., Wageningen, Netherlands). Our study explores how wild boar rooting relates to water erosion-related pedological parameters (phosphorus, Total Organic Carbon (TOC), pH and clay content). The amount of phosphorus was higher at the lower third of the slope than at the upper third of the slope, while pH was higher in samples collected from the upper third of the slope. Wild boar rooting caused notable differences in TOC levels between the rooting and the ring in both thirds of the slope. Phosphorus levels were lower in the rooting compared to the rings.

### 2.11. The Conservation Significance of the Karst Steppes in the Sofia Region (Western Bulgaria) as the Southernmost Refugium of the Steppe Biome in Europe: A Case Study of Ground Beetles (Coleoptera: Carabidae)

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During a study on the distribution and status of the protected steppe beetle *Carabus hungaricus* in the vicinity of the Sofia Basin (central-western Bulgaria), 42 sample plots were surveyed in the period between May 2021 and December 2023. Via 252 pitfall traps, 69,903 effective trapdays were realized, resulting in 15,333 ground beetle individuals (Coleoptera: Carabidae) from 184 species, of which about 30% were characteristic of the specific steppe biota. Local faunas were studied in terms of their taxonomic, zoogeographic, size, ecological and spatial structure. The main environmental gradients, investigated by using grouping, clustering and classification methods on carabid assemblages, showed the vital relevance of the anthropogenic influence on landscapes in the context of contemporary climatic conditions and changes. Additionally, we analysed the stenotopic steppe carabids for co-occurring species and microclimatic, phytocenotic and edaphic requirements. Four stenotopic steppe carabid species were recorded (*Carabus bessarabicus*, *C. hungaricus*, *Amara pastica*, *A. taurica*), of which one subspecies (*C. b. tangra*) is unique to the karst steppes of the Chepan Planina and Tri Ushi Mountains. The refugium found in these two mountains

represents the southernmost part of the entire Eurasian steppe biome and is unique both in terms of its location and the complex of specific steppe species forming distinctive communities, most of which have long since disappeared from the European Union. Along with the significant carabids found in this steppe complex, some other animals of conservation importance were established—*Bradyporus macrogaster*, from the Orthoptera, and Common spadefoot toad (*Pelobates fuscus*) and Ural field mouse (*Apodemus uralensis*) from the vertebrates. The conservation of this biota, unique to the Bulgaria and Europe steppe, requires an urgent declaration of the two mountains as protected areas and the identified steppe species and their habitats as priorities, along with their inclusion in national and European conservation documents with strictly protected statuses.

### 2.12. *Toxoplasma Gondii* as a Cause of Multi-Organ Failure in the Cat's Body

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This paper presents an analysis of a clinical case of toxoplasmosis in cats with multiple organ failure. A domestic cat at the age of six was admitted to the veterinary center of St. Petersburg. Earlier, the animal was diagnosed with feline immunodeficiency virus (FIV). It should be noted that a loss of appetite, depression, hyperthermia, labored breathing, coughing, and diarrhea became apparent after the cat had caught and eaten a bird that had flown into the dwelling a month earlier. A biochemical analysis of the animal's blood revealed hyperbilirubinemia (13.2  $\mu\text{mmol/L}$ ) and thrombocytopenia (74.0). An ultrasound examination revealed decreased intestinal peristalsis, enlarged mesenteric lymph nodes, hepatomegaly, and hyperemia. Radiologically, multiple small nodules were detected in the lungs. Pathologic foci were distributed bilaterally and asymmetrically, mainly in the lower parts of the lungs. A cytologic examination of the cat's bronchoalveolar mucus and peritoneal fluid revealed trophozoites of *Toxoplasma gondii*. DNA fragments belonging to *T. gondii* were identified using real-time PCR. The etiotropic and symptomatic therapies delivered to the cat under in-patient conditions were unsuccessful, and the outcome of the disease was fatal. In analyzing this clinical case, the following conclusions were made: *T. gondii* infestation cannot be excluded in the case of apartment-dwelling animals. The pathogen probably entered the cat's body by the cat eating a bird with *T. gondii* trophozoites in its organs and tissues. If a cat with a reduced immune status shows clinical signs such as enlarged lymph nodes, lethargy, decreased appetite, weight loss, nasal discharge, cough, intestinal disorders, a diagnosis of toxoplasmosis should be confirmed or excluded by serologic, PCR, or ELISA tests. Toxoplasmosis in animals with a reduced immune status can lead to severe systemic disorders, including damage to the digestive and respiratory systems, the liver, and other organs. The cumulative failure of several functional systems, developing as a terminal stage of the acute course of toxoplasmosis, can be fatal.

### 2.13. *Ant (Formicidae) Diversity of Olive Orchard Agroecosystems in Crete, Greece: The Effect of Agroecological Zone*

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Ants are among the most abundant insects in natural and agricultural ecosystems. In this study, for the first time, we investigated the ant fauna of olive orchards in Crete (Greece). Ants were surveyed with pitfall traps, placed in six paired conventional and organic olive orchards. Specimens were collected from October 2021 to July 2022. Orchards



were located in two agroecological zones (hilly and plain), three in hills and three in the plains. In each orchard, five traps were placed containing propylene glycol. Specimens were identified to the genus and species level using taxonomic keys. In total, 21 species and 14 genera were identified, out of a total of 28 recorded in Crete. The ants of *Aphaenogaster* were common and abundant in all the olive orchards, making this the most common genus. The species of the genus *Monomorium* also showed high numbers and distribution in all orchards. The small number of specimens belonging to the genera *Crematogaster*, *Lasius*, *Temnothorax* and *Solenopsis* is due either to their rarity in the olive orchards or to the fact that they are mainly arboreal species. The hilly olive orchards had a higher abundance of ants than the plain ones. Ants in olive orchards show high diversity and are important for pest regulation, soil fertility and nutrient recycling. Further analysis of the species found and of the effect of each farming practice on the ant diversity is required to determine which practices are the most suitable for maintaining an olive orchard's functional ant diversity and ecosystem services.

#### 2.14. Blood Testing as a Tool for the Conservation of Killer Whales (*Orcinus orca*)

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The species *Orcinus orca* is spread across the globe. As top predators, killer whales constitute an important part of global biodiversity. Currently, they are not hunted. However, killer whales sometimes strand, experience trauma, become stuck in the ice, etc. Human aid is essential to save them. A case of a female Springer who was successfully returned to the wild provides an encouraging example. Therefore, every effort to improve marine mammal medicine is valuable.

Ten juvenile killer whales spent a year (summer 2018–summer 2019) in human care in Srednyaya Bay, Vostok Gulf, in the Sea of Japan (Russia). The animals were clinically healthy. Blood samples were taken regularly to monitor their health state as a part of the veterinary routine. In total, about 250 blood samples were taken and processed using clinical and biochemical blood test protocols. These data were also analyzed retrospectively to find out what kind of influencing factors could have been related to their blood analytes.

In our results, we found that the temperature of the sea water, period of time spent in captivity, and individual characteristics affected most of the blood analytes, such as the red blood cell count; total white blood cell count; absolute segmented neutrophil count; absolute lymphocyte count; absolute eosinophil count; neutrophil-to-lymphocyte ratio; and more than 20 biochemical analytes.

Thus, a unique data set on their blood analytes was accumulated. These data can be applied to achieve the most accurate veterinary aid possible for killer whales when needed.

#### 2.15. Wildlife Fences to Mitigate Human–Wildlife Conflicts in Africa—A Literature Analysis

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The deployment of wildlife fences in Africa serves as a critical intervention to balance the needs of wildlife conservation with human safety and agricultural productivity. This review synthesizes current research and case studies to provide a comprehensive understanding of the implications, benefits, and drawbacks of wildlife fencing in Africa. The

information used in this review has been drawn from 47 articles that were selected after a thorough search on Google Scholar, Web of Science, and Scopus. The results indicate that the main reason for fencing is the mitigation of human–wildlife conflicts. The electric fence is the most common type and is prominently used to protect agricultural lands from crop-raiding species, thereby enhancing food security and reducing economic losses for rural communities. Elephants have been mentioned as being the most targeted for wildlife fencing. They also cause the most damage to the fences and pave ways for other species to escape from protected areas. Antelopes and large carnivores are also common targets for wildlife fences. Fences were found to be effective if well maintained through frequent inspections for damage and permeability. Several authors have documented the difficulty in fencing in primates, and burrowers or high-jumper species like the leopard. Fences are also costly to install and maintain, which has great economic implications on the local communities living near wildlife conservation areas. Despite their benefits, wildlife fences also pose ecological challenges, such as habitat fragmentation and restricted animal movement, which necessitate integrated management approaches that include the consideration of wildlife corridors and crossing structures. Our results offer insights for policymakers and conservationists to optimize the use of fences in diverse environmental contexts of the African continent.

#### 2.16. Assessment of Oxidative Damage in Red Blood Cells of Seabirds from Argentina

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Sublethal effects caused by exposure to contaminants can reduce fitness in seabirds and ultimately affect their populations. Red blood cells are particularly susceptible to oxidative damage caused by certain pollutants such as oil. This damage can result in injury to hemoglobin, which aggregates and can be observed as inclusion bodies within erythrocytes, called Heinz bodies. The aim of our study was to determine the hematological biomarkers of oxidative damage in seabirds to evaluate its possible effects on the birds' health. Blood samples were taken from nine specimens of seabirds admitted to Aquarium Mar del Plata in Buenos Aires Province, Argentina. Eight Magellanic penguins (*Spheniscus magellanicus*) and one black-browed albatross (*Thalassarche melanophrys*) were sampled. Blood was collected in tubes with EDTA for the determination of reticulocytes and in capillary tubes for the determination of hematocrit. Blood was incubated with brilliant cresyl blue, and smears were made for the quantification of reticulocytes and Heinz bodies. In both cases, the number of cells/1000 mature erythrocytes was calculated. Hematocrit and reticulocyte values were within the normal range for birds. The presence of Heinz bodies was not observed in any of the birds. Additionally, no anomalies in these biomarkers were observed in two birds with signs of fuel on their feathers, but without signs of oil. This is in agreement with what was found for other bird species, where the values of Heinz bodies in birds from places not impacted by oil were zero or close to this value. Our results constitute the first evaluation of these markers in these two species of seabirds. Although biomarkers related to oxidative damage to red blood cells were not found to be increased, the information presented in our study allows us to establish baselines for the evaluation of future impacts associated with human activity.

### 2.17. Strategies and Challenges in Biodiversity Conservation

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**Introduction:** Biodiversity conservation is critical for maintaining ecosystem services and ensuring the resilience of natural environments. Despite numerous conservation efforts, biodiversity loss continues at an alarming rate due to anthropogenic activities, climate change, habitat destruction, and pollution. This review aims to analyze the current strategies, challenges, and future directions in biodiversity conservation. **Methods:** A comprehensive literature review was conducted using peer-reviewed journals, government reports, and NGO publications. Key conservation strategies were identified and evaluated based on their effectiveness, scalability, and sustainability. The analysis also included case studies of successful and unsuccessful conservation programs globally. **Results:** This review highlights several effective strategies, such as protected areas, community-based conservation, habitat restoration, and biodiversity offsets. The role of international agreements, like the Convention on Biological Diversity (CBD), and national policies in conservation efforts were also assessed. However, challenges such as inadequate funding, lack of local community engagement, and insufficient enforcement of conservation laws were identified as significant barriers to success. **Conclusions:** To enhance biodiversity conservation, it is crucial to adopt an integrative approach that combines scientific research, policy making, and community participation. Emphasizing adaptive management practices and leveraging technology for monitoring and enforcement can address some of the current challenges. Future conservation strategies should also focus on building resilience against climate change and promoting sustainable development.

### 2.18. Investigating the Potential of Mushroom Mycelium Textile Blends

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Sustainable and renewable resources such as mushrooms and mushroom composites offer a wide range of application possibilities in different areas. In particular, the combination of mushroom mycelium and textiles shows promising approaches for the filtration of wastewater in the textile industry, air purification, use in protective clothing, medical applications such as blood purification or use in packaging and even in building construction. Mycelium, the vegetative network of fungi, is an alternative and renewable material that is easy to cultivate. In addition, many organic wastes from the agricultural industry can be used as nutrients for mycorrhizal cultivation, further increasing sustainability. In addition, fungi have anti-inflammatory, immunomodulatory, antibacterial, antifungal, antitumor, antioxidant, antidiabetic and lipid-lowering properties that can be exploited for specific applications. Despite several studies being conducted in the last few years, the use of mycelium as a textile alternative has not been extensively explored. In the present study, mycelium of the fungus *Pleurotus ostreatus* was used to produce mushroom–mycelium textile composites, and their mechanical properties were investigated. The aim of this study was to evaluate the potential of mycelium in the textile industry and to identify possible applications. The results of this study should help promote the use of mushroom mycelium in textile production and at the same time develop innovative, environmentally friendly materials that meet the increasing demands for sustainability and resource conservation.

### 2.19. Revealing Participants of Amphibian and Reptile Mapping Citizen Science Project in Hungary

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Amphibian and Reptile Mapping is one of Hungary's pioneer citizen science initiatives contributing to nature conservation and scientific research. Through the available interfaces, herpetofauna observations are shared by volunteer observers. However, there is still a shortage of knowledge regarding these participants. To achieve the dual benefits of the project for participants and scientists, in this study, we aim to establish a better understanding of volunteers' experiences when participating in Amphibian and Reptile Mapping. In 2023, an online questionnaire was conducted to explore volunteers' experiences, including demographics, data uploading, technology use, knowledge gain, and changing attitudes and behavior. From the 182 valid responses, descriptive statistics, the chi-square test ( $p < 0.05$ ), Cramer's V, and the Fisher–Freeman–Halton Exact Test were used to determine variable associations. Our findings show more than two-thirds of participants have higher education experience (69%). The majority prefer the website for uploading observations (65%) and making records occasionally (68%). The most frequently observed amphibian species was *Rana dalmatina* (14%), followed by *Bufo viridis* (12%), and the reptile species were *Lacerta viridis* (13%), *Zamenis longissimus* (11%), and *Emys orbicularis* (10%). Less than half of the respondents (44%) stated that they acquired knowledge through participation in the project. No association was found between the participation period and knowledge gained. Motivations for participation, such as environmental responsibility, personal interest in herpetofauna, and learning desire, were associated with knowledge gain. A significant association was observed between the upload frequency and work or educational environments related to nature conservation. More than half of the respondents (54%) showed an attitude change due to the project. The participant's motivations for recreation, environmental responsibility, and learning desire were associated with attitude-behavioral change. We recommend developing strategies that enhance participation experience by increasing the knowledge gain and attitude changes in participants.

### 2.20. The Introduction of Honey Bees for Reducing the Biodiversity of Pollinators

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The gene pool of endemic honey bees ensures the conservation of the number of bee colonies. In Russia in the last century, *Apis m. mellifera* L. (60%), *A.m. caucasica* Gorb. (12%), *A.m. rempries* (5%), *A.m. carpatica* Avet. (18%), and Far-East bees (5%) were mainly bred—the number of bee colonies was 10 million. In modern beekeeping, as a result of mass introduction, there has been a change in the gene pool of honey bees and an increase in the death of bee colonies, which entails a reduction in pollinators. This research was conducted between 2000 and 2024, in which a survey of 1.5 thousand beekeepers was conducted, and 560 apiaries from 56 regions were surveyed. The assessment of honey bees by morpho-phenotypic and biological characteristics was carried out according to Alpatov (1948) [15] and Ruttner (2006) [16]. Currently, there are three million bee colonies in Russia: *A.m. mellifera* L. (4%), *A.m. caucasica* Gorb. (2%), *A.m. carpatica* Avet. (12%), Far-East bees (5%), new breeds of Carnica 66%, Bacfast 4%, and 11% bees of unknown origin. With

the advent of new breeds, in the last 20 years there have often been collapses in apiaries. Reasons: 1. the use of pesticides; 2. up to 30% of death is as a result of a cold long winters; 3. the appearance of new diseases, for example, Tropilelapsosis; 4. the import of bee bags with broods, and the import of new populations of Varroa mite with varying degrees of drug resistance. The diversity of honey bee populations based on their genetic nature is a unique and irreplaceable resource for the creation and reproduction of new subspecies of bees resistant to pests, diseases, and changing environmental conditions.

#### 2.21. Standardization of Long-Term Pollen Cryopreservation of *Vigna vexillata* to Facilitate Wide Hybridization

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*Vigna vexillata* (L.) A. Rich., known as tuber cowpea, Zombi pea, and wild cowpea, is a legume that thrives in diverse climates and shows resilience against bruchid and abiotic stress. *V. vexillata* is a close relative of the cultivated cowpea, and it is cultivated for its storage roots, protein-rich seeds, forage, and erosion control capabilities. It grows in both protected and partially disturbed habitats in hilly and sub-hilly regions of peninsular India and the Himalayas. The cryopreservation of pollen grains is a vital technique for conserving important alleles and making genetic resources readily available for breeding programs. This method overcomes the challenge of asynchronous flowering, facilitating the creation of hybrids with pest and disease resistance, high yield, and desirable qualities. To advance wide hybridization studies, pollen cryopreservation was evaluated across 12 accessions of *V. vexillata*. Fresh pollen viability was recorded between 85.72% and 93.56%. This study assessed pollen viability under different storage conditions (25, 4, –20, and –196 °C) and various durations (1, 3, 5, 7, 9, and 24 h; 1, 2 weeks; and 1 month). Optimal long-term cryopreservation was achieved with a pollen moisture content of 10–12% following a 5-min desiccation period in a laminar airflow chamber. A significant negative correlation was observed between pollen viability, storage duration, and temperature (25, 4, and –20 °C) across all accessions. Pollen stored at –196 °C demonstrated significantly higher viability, ranging from 81.14% to 93.07%, compared to other storage conditions. The established cryopreservation protocol successfully maintained pollen viability for up to one month and enabled effective pollination, fruit development, and seed set in crosses using cryopreserved pollen.

#### 2.22. Carabidae Diversity in Olive Orchards in Crete, Greece. The Effect of Agroecological Zone and Management System

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In the island of Crete (Greece), olive orchards are the most common tree crop. The microclimate, but also the landscape, has an overall effect in the functioning of the olive agroecosystem. Factors related to olive orchard management, climate and landscape contribute substantially to the diversity of indicator organisms such as coleoptera. Carabidae are one of the best bioindicators for the protection of ecosystems. Carabidae beetles are important, because they are sensitive to anthropogenic interventions, such as the use of insecticides and heavy metal accumulation in agroecosystems. Carabidae fauna of olive orchards was for the first time investigated in Crete. During the period of October 2021–October 2022, we surveyed carabidae beetles on a monthly basis, using pitfall traps, in 18 olive orchards under different management systems (organic, conventional, abandoned) located across two agroecological zones, i.e., hilly and plain. In each orchard, five traps were placed containing propylene glycol. We found 14 different genera and identified 12 species. The most common species were *Carabus banoni* and *Tapinopterus creticus*. Olive orchards in the hilly zone presented higher populations than the plain ones, while higher populations were found in the organic orchards. The differences in richness between agroecological zones appear to be a result of altitudinal differences in relation to air temperature and humidity, although this requires further investigation. The geographic proximity of the studied fields hampers strong differences in species composition. This is a first attempt to understand the impact of human (agricultural) activities on carabidae beetles in the Mediterranean region. We highlight the importance of organic olive orchards as sites of increased carabidae diversity. Further research is required to evaluate carabidae as potential indicators for assessing the impact of agriculture on biodiversity.

### 2.23. Strategies for Conservation of Biodiversity: A Synergistic Approach in Designing Landscapes, Population Management and Reforestation

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In order to achieve adequate conservation of biodiversity in human-modified landscapes, there is a need to introduce proper measures that balance ecological requirements with socio-economic realities. This abstract emphasizes three distinct perspectives in the conservation of biodiversity: landscape management, integrated conservation strategies (including in situ and ex situ conservation), and reforestation. The first strategy promotes designing suitable landscapes to have a significant amount of forest cover (>40%), which should be structured into large-scale and dispersed small-scale patches enclosed within a high-quality matrix. Designing such landscapes offers a balance between development and conservation objectives, protecting species that live in forests while fulfilling human requirements. The second approach, “One Conservation,” deals with a comprehensive framework to integrate in situ and ex situ conservation as a driving factor in restoring ecosystems. This can be achieved by facilitating the exchange of genetic material among in situ and ex situ populations and developing new reproductive biotechniques that serve as great tools for restoring species to their natural surroundings. Another approach evaluates reforestation as a critical tool for the conservation of biodiversity. Reforestation should be implemented in tropical regions like Brazil and Indonesia, where reforestation-able areas overlap with habitats of endangered species, to attain conservation on a global scale. On the other hand, this strategy delves into understanding the reduced impact of reforestation on climate change while making the cost and efforts of reforestation reliable to promote economic incentives. Integrating these measures with one another contributes greatly, not just in biodiversity conservation but also in achieving sustainability, paving the way for resilient ecosystems in this constantly changing world.

#### 2.24. A Comparison of Filtrate and Sediment Samples of Cladocera in Hungarian Shallow Lakes to Characterise the Types of Wetland

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Wetlands are essential parts of the ecosystems that sustain plant and animal life on Earth. Water fleas, also known as cladocera, are microcrustaceans, or microspecies, with bodies that are translucent, plate-like appendages with a length of less than one millimetre. They are filter feeders. These microscopic crustaceans feed on phytoplankton and serve as a source of food for aquatic zooplankton. Cladocera species found in various lake zonations may vary based on elements such as the water quality and macrophytic organisms present in the ecosystem. Through wetland modification and degradation, recent anthropogenic activities are having a significant negative impact on the quality of the wetland and habitat. Wetlands need to be closely observed and evaluated to yield data that will be useful for restoration efforts. The purpose of this study is to determine whether the filtrate samples obtained from the water samples will reveal any cladoceran remnants in the sediments. This will also be achieved through examining the characteristics, both chemical and physical, of the sediment samples from Cladocera. A comparison between the sediment Cladocera fossils and the filtrate samples will provide helpful information about whether the sediment and filtrate samples show the same occurrence or quite different. The findings will contribute to our understanding of the patterns of biological community, distribution, and sediment analysis as a functional tool for bio-identification of the status of wetland quality. A comparison between the sediment and filtrate samples may be useful as a biological indicator tool to track how species are responding to changes in their natural or altered habitat. Multivariate statistical approaches will be used in this study for multiple variables.

#### 2.25. New Data on Earthworms in Two Nature Conservation Areas in Hungary

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Regardless of their well-known role in providing ecosystem services and maintaining soil quality, data on earthworm diversity are sporadic. There have been multiple efforts to collect samples, but it is obvious that Hungarian protected areas are not under thorough investigation. This research provided new information for earthworm research on a slope of the Gödöllő Hillside and at the border of a peaty meadow near Galga Creek. The Gödöllő area is steep while the Galga Creek area is a wetland with a sandy area. We hypothesize that there will be more earthworms at the bottom of the slope, being a more favorable habitat due to the more abundant food sources, while in the Galga Creek area, a higher number are expected to be present around the sandy "hill", as the peaty meadow provides a much more favorable area compared to the sandy hill, because sand is known to dry out easily and there is less organic matter, so it is not favored by earthworms (being the opposite of the peaty area). Earthworms were collected in five replicates of a 25 × 25 × 25 cm hole dug using a shovel. The search normally takes 30 min (gross: including the digging, counting,



and recultivating the area, as well as marking the bottles holding the earthworms and collecting samples for soil analyses). *Aporrectodea rosea* was found at both sites. *Eisenia fetida* and *Lumbricus terrestris* were only found in the peaty meadow site while *Octolasion cyaneum* was only found in the steep slope area. Both sampling sites (the hilly area and the peaty meadow) proved the hypothesis: the lower section of the steep slopes and the less sandy area of the peaty meadow provided better habitats, and this was associated with higher earthworm abundance and earthworm weight. The research provides a baseline for further investigations because there is a lack of related publications.

#### 2.26. The State of Three *Cypripedium guttatum* (Orchidaceae) Populations in the South of Western Siberia (Tyumen Region)

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The family Orchidaceae is globally recognized as one of the richest and most diverse plant families. Orchids inhabit a variety of habitats, and many of them are endemic, rare and/or threatened taxa. Therefore, these plants are of high conservation interest. In Russia, the Orchidaceae is represented by 135 species and 13 subspecies, and their number varies among administrative regions. In the south of Western Siberia, 25 species of Orchidaceae are included in the Red Data Book of the Tyumen Region, including *Cypripedium guttatum*, which has category 3 (rare species). This species has been known in the Tyumen Region since the early 20th century. However, almost no special population studies have been published previously for this orchid, with an exclusion of 20-year-old-dated data from conference proceedings, which, unfortunately, are not available online for a wide audience. In this study, we conducted population studies of *Cypripedium guttatum* in three localities to gain an understanding of its status in Western Siberia. In each location studied, the morphometric parameters of individuals were measured, and the population age structure and the density of individuals were determined. The flora that accompanied *Cypripedium guttatum* was recorded in each location. The data obtained can be used as a basis for future assessment of the species according to the categories and criteria of the IUCN Red List, and, consequently, the creation of the Red List of Vascular Plants of the Tyumen Region (Western Siberia).

#### 2.27. Comparative Analysis of Natural and Artificial Light Perception in Three Moth Species

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Light pollution is a growing concern affecting biodiversity, especially moths, which are crucial pollinators. This study evaluates the similarity between artificial and natural light using the Daylight Similarity Index (DSI) and Moonlight Similarity Index (MSI). These indices are then weighted by the visual responses of three moth species: *Cydia pomonella*, *Grapholita molesta*, and *Lobesia botrana*; and *Homo sapiens*. The study analyzed 193 spectra from LED lamps and compared them to the D65 daylight spectrum and the full moon spectrum.

The results indicate that the maximum similarity occurs at correlated colour temperatures (CCTs) between 3000 and 4000 K, with and without weighting. Higher CCTs are perceived as more similar to natural light by insects. Interestingly, despite their different flight periods, the three moth species did not show significant differences in light perception, suggesting that visual perception mechanisms are more related to family than lifestyle. A slight shift towards diurnal vision was observed in crepuscular species, hinting

at a trend towards diurnal vision. This research highlights the importance of considering insect vision in designing lighting systems to mitigate light pollution's impact on moths. Further research is recommended, particularly with UV-inclusive light and other light characteristics, to develop more effective strategies for managing pest populations and protecting biodiversity.

#### 2.28. Conservation Translocation: A Crucial Strategy to Protect Endangered Plants

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Plant translocations are increasingly crucial in conservation efforts, establishing new populations, augmenting declining ones, and reintroducing species to their native habitats. Despite their effectiveness in reducing the risk of extinction for seriously threatened plants, translocations are often the last option due to high costs and failure risk. However, such actions are urgent in the Mediterranean Basin, a global hotspot for plant diversity, which faces significant anthropogenic pressures and climate change. In this context, *Dianthus morisianus* Vals. (Caryophyllaceae) stands out as a narrow endemic species in a coastal dune system in south-west Sardinia. Its habitat is in continuous decline; consequently, it is assessed as Critically Endangered on the IUCN Global Red List (CR), yet it is not protected by any international, national, or local regulations. To reduce the extinction risk of *D. morisianus*, three conservation translocation actions have been undertaken since 2010. The first, in November 2010, involved the construction of protective fences and the reintroduction of 113 juvenile plants. The second, in February 2012, introduced 25 juvenile plants to an open, unprotected site. The third, in March 2018, aimed to strengthen the population by introducing 38 adult plants. Long-term monitoring over 14, 12, and 6 years, respectively, indicated high survival, flowering, and fruiting rates for fenced plants. The newly fenced populations had a survival rate exceeding 96% in the first year, reaching 100% in subsequent years. Flowering and fruiting rates ranged from around 40% in the first year to over 70% in the following years. Many new seedlings appeared after two years, increasing annually (quintupling over time). In contrast, the unprotected site exhibited low seedling numbers and no reproductive plants. Fenced populations demonstrated enhanced long-term survival and reproductive success, becoming self-sustaining through successive generations. This translocation project showcases an effective strategy for reintroducing endangered species and offers a model for similar conservation efforts in Sardinia and the broader Mediterranean region.

#### 2.29. Ethno-Botanical, Ethno-Medicinal and Ex Situ Conservation Practices of *Prunus ceylanica*: An Endangered Tree Species from Andhra Pradesh, India

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*Prunus ceylanica* (Wight) Miq. is an evergreen tree of the family Rosaceae that is native to India and Sri Lanka and is commonly called ceylon cherry. The species of this nomenclature type was collected from the Ceylon region. Ethno-botanical data on *Prunus ceylanica* were collected by conducting a field survey in tribal communities of the forest area between August 2022 and July 2023. The ethno-botanical data were collected from the tribal communities by field survey, open interviews with traditional healers, and questionnaires provided to the tribal populations in the forest area. The whole plant is used in the traditional system. The traditional usage of the plant by indigenous tribal communities was collected by interacting with the local people. Different parts are used in curing skin diseases, boils, and stomach ulcers. The fruits are eaten by jungle cats. *Prunus ceylanica* is an ethno-botanically useful plant. The wood is moderately hard and used in making raw furniture, wooden boxes, planks, and booths. The wood material is a good source of fuel for firing bricks. *Prunus ceylanica* is medicinally used in various

ways by local tribal communities. When squashed, the bark, leaves, and fruits emit the smell of bitter almonds. Potent applications of the tree are documented. *Prunus ceylanica* was conserved by some ex situ conservation practices like seed collection and saplings protection. The seeds were collected from the tree and sown in poly bags, achieving a good number of saplings. Because it is endangered, conservation of *Prunus ceylanica* was carried both onsite and offsite to increase the number of individuals. Stem cuttings show moderate success with the application of hormones. The present study focuses on the ethno-botanical utilization and potential application of *Prunus ceylanica* from the maredumilli forest region of Andhra Pradesh

### 2.30. Comparative Evaluation of Two Cryopreservation Protocols with Programmable Freezing of Drone Semen

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**Introduction:** The main purpose of the cryopreservation of drone semen is to make possible its use for the artificial insemination of queen bees to enable reproductive abilities on the same level as in bees inseminated by fresh semen. The most widespread methods for the cryopreservation of drone semen are freezing in liquid nitrogen vapor and programmable freezing. The most widespread form of cryopreservation for honeybee semen is using straws. However, the impact of liquid nitrogen on a sample in a straw is larger, as the surface area of the sample is increased. The purpose of this study is to comparatively assess both straws and cryovials in the programmable freezing of drone semen. **Methods:** The survival rate of spermatozoa was assessed according to overall motility and membrane integrity (viability) through a staining method using 1% eosin solution. **Results:** During the comparative analysis of the cryopreservation forms studied, it was revealed that cryovials are credibly superior to straws in programmable freezing at a rate of  $1-3^{\circ}/\text{min}$ , as shown by the overall motility ( $U = 3166.5-3637$ ,  $p < 0.05$ ) and viability ( $U = 2786.5$ ,  $p < 0.05$ ) of spermatozoa. The cryopreservation conditions influenced the quality of frozen-thawed semen. The overall motility of spermatozoa frozen in cryovials and straws at a rate of  $1^{\circ}/\text{min}$  was shown to be credibly higher in comparison with samples frozen at a rate of  $3^{\circ}/\text{min}$  ( $t = 2.3$  and  $t = 2.9$ ,  $p < 0.05$ ). **Conclusions:** As such, the cryopreservation of drone semen in cryovials has a manifest advantage over those using straws, in particular with a slower programmable freezing rate of  $1^{\circ}/\text{min}$ .

### 2.31. Effect of Domestic Cats on the Behavior of Common Birds in the Peri-Urban Area of the City of Granada

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Invasive alien species such as the domestic cat (*Felis silvestris catus*) have remained linked to humans for millennia, initially as commensals and currently as companion animals. In urban environments, their populations increase due to the care provided by citizens, which can have a negative effect on the biodiversity concentrated in parks and gardens. To see the effect that the presence of cats has on birds, fixed observation points were chosen in areas with historic gardens in the city of Granada (Spain), such as the Alhambra monumental complex, a place where a feline colony is managed through the TNR method. Five-minute counts of both bird abundance and richness were performed. Cats were more abundant on average in areas where there were feeding points, and depending on the area (with or without the presence of feeding points), the abundance of cats had a

significant effect on the abundance and richness of birds in the studied area. In areas near the feeding points, the abundance and richness of birds were lower, and the number of cat contacts present in the area was greater ( $3.17 \pm 0.32$ ; range 0–12 cats), while in the areas where there were no feeding points, bird abundance and richness were greater, with a lower number of cat contacts ( $0.14 \pm 0.02$ ; range 0–2 cats). This work highlights the negative effect of cat feeding points and the abundance of cats on birds. A possible management measure in the monumental complex could be to feed the cats of the feline colony at a single (or a maximum of two) feeding point, located in one of the areas with the greatest influx of tourists, with little vegetation, thereby reducing the impact that both domestic cats and tourist overcrowding have on them.

### 2.32. Biodiversity of Carabid Beetles in Different Types of Forest in the Region of Abruzzo, Italy

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This study examines different forest types and biodiversity characteristics in the Abruzzo region (Central Italy), within a landscape delineated by diverse large forests. Historically, agro-pastoral activities have altered the region's mountains ecosystems, leading to a decrease in forest cover and associated species. A significant reforestation campaign was run from the middle of the 19th century to the end of the 19th, primarily using italic black pine, aimed to remedy these changes. This research focuses on assessing the biodiversity of different forest types: oak, beech, and conifer compared by the diversity of Carabidae, Coleoptera. Carabid beetles were selected as bioindicators due to their sensitivity to environmental changes and ease of sampling. The study involved placing pitfall traps in various forest types to collect and determine Carabidae diversity in each habitat. This study, which should highlight the importance of these often-neglected taxa in maintaining ecosystem health, also aimed to integrate arthropod conservation into ecosystems management activities and into rewilding dynamics studies. The results of this study are preliminary. It is the first time that forests of this region were explored in that way, studying the combination of the vegetation with forest structure and related carabid species. This study fits into a broader context of studies dedicated to these issues involving various taxa from the various kingdoms of living beings.

In our study, a Generalized Linear Model (GLM) will be used to analyse the relationship between beetle Richness and Abundance and the various forest characteristics (i.e., forest types and structure, necromass, vegetation and estimated age). GLM will also be used to analyse the activity/density ratio, to provide a more accurate representation of beetle populations, since richness and abundance can be biased by the degree of activity of certain species.

### 2.33. Tree Planting in Ibadan Metropolis as an Indication of Climate Change Mitigation

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Urban tree planting is a vital strategy for mitigating the effects of climate change, particularly in rapidly urbanizing areas like Ibadan, Nigeria. This study addresses the challenge of urban heat islands and air pollution, exacerbated by increasing deforestation and inadequate green spaces. The study aims to understand the determinants influencing residents' adoption of tree planting practices. Using a cross-sectional survey design, data were collected from 450 households across five urban local government areas (South-West,

North, Akinyele, North-East, and South-East) with the use of structured questionnaires which assessed awareness, perceptions, and factors motivating tree planting in Ibadan metropolis. The findings revealed a strong awareness of climate change, with residents citing benefits like shade, improved air quality, and aesthetic enhancement as key motivators for planting trees. A logistic regression analysis identified significant predictors, including environmental awareness and access to resources, which positively influenced participation in tree planting activities. This study concludes that enhancing public education and providing accessible resources are critical for increasing urban forestry participation. This implies that community-driven actions are pivotal in promoting sustainable urban environments and mitigating climate change. It is therefore recommended that targeted campaigns and government support should be enacted to increase tree planting, thereby enhancing urban resilience to climate change.

#### 2.34. Do Weight Loss Drugs Affect the Survival and Productivity of Aquatic Invertebrates?

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The increasing use of pharmaceutical drugs in medicine, agriculture, and domestic animals could increase active pharmaceutical ingredients (APIs) in the environment. The accumulation of APIs in the environment could present an insidious threat to biodiversity, as APIs and their metabolites impact food webs. We assessed the potential effects of a widely used API, orlistat, on aquatic invertebrates. Orlistat, an active ingredient in weight loss drugs, interferes with the digestive enzymes that help breakdown lipids. We reasoned that orlistat could interfere with the assimilation of lipids in aquatic organisms and affect growth, reproduction, and survival. We selected *Daphnia magna*, a primary consumer, as a model organism for aquatic invertebrates. The experimental treatment involved orlistat dissolved in ethanol added to natural spring water; the control treatments (12.0  $\mu\text{L}$  EtOH/L  $\text{H}_2\text{O}$ ) were the same but without the orlistat. In replicated experiments, the survival of *Daphnia* in orlistat (~2  $\mu\text{g}/\text{mL}$ ) was less than that of controls ( $p \leq 0.001$ ). There was also an effect on productivity. We observed delayed egg development, failed egg development, and a lower frequency of neonate births in the orlistat treatment. This suggests that females in the orlistat treatment allocated less of their endogenous resources to reproduction. We also noted subtle differences in growth. The rate of body molts appeared to slow down in the orlistat treatment. We also noted a dose-dependent effect on survival when we increased the concentration of orlistat from 2.38 to 6.95  $\mu\text{g}/\text{mL}$ . Although orlistat is hydrophobic, we suspect that it could accumulate in the surface layer, the edges of the littoral zone, and in sediments. We conclude that orlistat can affect aquatic invertebrates. Further research is needed to determine the potential impacts on aquatic food webs and the potential threats to biodiversity.

#### 2.35. A Native Potato Collection from the Geographical Department of Amazonas (Peru)

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Native potatoes (Solanaceae) are an important Andean crop due to their excellent culinary quality, good flavor, and floury texture. They also contain vitamins, protein, minerals, and carbohydrates. In Peru, it is possible to observe an abundant morphological

diversity of native potatoes grown in the Andean region. Native potatoes are cultivated and conserved mainly by old peasants in the highlands of the geographical department of Amazonas in Peru. However, this crop is disappearing due to the lack of knowledge of many farmers, limited studies on native potatoes, and climate change. To close this gap, as a first step, collecting trips in potato-producing localities in the geographical department of Amazonas were conducted in 2023 and 2024. These expeditions resulted in the collection of 96 native potato accessions as tubers from three provinces and 10 districts from Amazonas. This genetic material is currently under a regeneration process in the greenhouse of UNTRM. Then, DNA will be extracted from young fresh tissue, and these will be shipped for whole-genome sequencing. A morpho-agronomic evaluation of this germplasm will be conducted in two locations in the present growing season, and a new cycle of evaluation will be performed in the following season. Genetic diversity parameters and population structures will be estimated from an SNP panel, and together with the morpho-agronomic data, a genome-wide association analysis will be carried out. This is the first comprehensive expedition of potatoes in the Amazonas. In the near future, genomic prediction models will be evaluated for enhancing the selection of accessions within this UNTRM potato germplasm, incorporating top genotypes into breeding programs. We expect that these efforts will stimulate additional work on potatoes in other geographical departments in the Peruvian Andes, alleviating poverty in rural areas.

### 2.36. A Review of Seaweeds in Brazilian Lagoon Environments

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Lagoons occupy 13% of the planet's coastal area, provide various ecosystem services, and are among the most threatened ecosystems in the world, mainly due to the intense urbanization of their surroundings. Macroalgae are efficient bioindicators used worldwide for monitoring aquatic environments. In Brazil, studies on macroalgae are concentrated in marine regions. The objective of this work was to conduct a systematic review of studies on marine macroalgae in Brazilian lagoons. Using Google Earth, the lagoons were located and mapped to assess national patterns. Between September 2021 and March 2022, searches were conducted in five databases in all languages, using the following keywords: (\*alga\* OR seaweed) AND (lagoon) AND (Brasil\* OR Brazil\*). Our results identified about 290 lagoons on the Brazilian coast. The Rio Grande marine ecoregion had the highest number of lagoons (n = 125), followed by the northeastern and southeastern ecoregions. Of the 17 coastal states, only four lack lagoons. This systematic review cataloged 36 studies on macroalgae conducted in only 11 lagoons, identifying 216 taxa, with 57% being Rhodophyta, 32% Chlorophyta, and 11% Ochrophyta. Lagoa dos Patos and Araruama had the highest number of studies. The use of these organisms as bioindicators is essential for monitoring the ecological status of these environments. It is recommended that a greater number of lagoons be the focus of long-term monitoring programs such as PELD. The natural conservation of coastal lagoons is important not only for their ecological significance but also for the valuable ecosystem services they provide for human well-being.

## 3. Marine Diversity

### 3.1. In Silico DNA Barcoding by the COI Gene of *Crassostrea virginica* in the Atlantic Ocean and Caribbean Sea

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**Background:** “*Crassostrea virginica* belongs to a class of bivalve mollusks that play vital roles in the marine ecosystem. This species holds economic and biological significance, making it a key species in the Atlantic, Caribbean, and Gulf of Mexico. This study aimed to understand the genetic diversity of *virginica* by barcoding techniques and if the distribution of oysters in the Atlantic Ocean is known, it would indicate which areas require further study.

**Methods:** Two thousand and eight sequences of *virginica* COI gene were downloaded from BOLD, aligned, and trimmed using BioEdit and ClustalW. For the haplotypes and their networks, MJN used Network and PopArt. DnaSP was used for sequence grouping and population genetic analysis. The p-Distance and Tajima-Nei values were obtained by Mega v11 software. Finally, to generate the phylogenetic tree, IQTree using maximum likelihood and FigTree were utilized.

**Results:** The three most abundant haplotypes were AY5422 (58.937%), GB8915 (9.661%), and GB1513 (5.314%). Four sets of sequences have been generated by the origin of capture: 176 for the United States, 9 for Canada, 17 for Cuba, and 4 for Panama. The results revealed two haplotype networks based on region and frequency. A total of 42 different haplotypes. The haplotype network showed that they were grouped by regions, with Canada and the US mixed, as well as Cuba and Panama. Genetic diversity values were nearly zero for individuals within the same geographic populations, while there were higher values among the four regions described (0.01 FST 0.9). The Tajima-Nei analysis revealed values ranging from approximately 0.001 to 0.029 within regions and between regions, respectively.

**Conclusions:** This study revealed that among the 4 regions examined, there were significant genetic differences, indicating the need to explore additional regions in the Gulf of Mexico to develop conservation and commercialization initiatives for *virginica*.

### 3.2. Dynamics of *Lingulodinium polyedra* Development in the Bulgarian Part of Black Sea (1992–2022)

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Long-term data on the phytoplankton of the Bulgarian Black Sea (BBS) coast describe three states for the ecosystem: a “pristine” reference phase (1954–1970 years); an intensive anthropogenic eutrophication (1970–1991 years) phase; and a post-eutrophication phase after the early 1990s of the 20th century. The eutrophication period is characterised with ecosystem degradation and intense phytoplankton blooms, some of which are formed by the potentially toxic species *Lingulodinium polyedra*. This warm-water species is a red tide former that has been associated with fish and shellfish mortality events. In the 1980s, *L. polyedra* reached a highest biomass of 84.4 g·m<sup>-3</sup> in Varna Bay, BBS. The aim of this long-term study (1992–2022) was to provide a comprehensive overview of the development of *L. polyedra* in the phytoplankton biocoenosis in the Black Sea when under anthropogenic stress and during the ecosystem recovery. An analysis of the distribution of the dinoflagellate *L. polyedra* is based on a total of 5126 phytoplankton samples collected for the period 1992–2022 under projects led by the Institute of Fish Resources, Varna. Samples was analyzed by standard methods validated for the Black Sea, and phytoplankton abundance and biomass were determined. The species are most abundant in summer in coastal marine areas exposed to anthropogenic influence. During the analysed period, decreases in the abundance and biomass of *L. polyedra* in Bulgarian Black Sea waters were observed. We

established that the frequency of the occurrence of *L. polyedra* was higher in the first half of each decade of the period and was related to changes in hydrometeorological conditions in the Black Sea.

### 3.3. Sponges of the Order Poecilosclerida (Porifera) from the Burdwood Bank and the Scotia Arc, SW Atlantic Ocean

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The Burdwood Bank (BB) hosts a Marine Protected Area in the SW Atlantic Ocean, situated within the Scotia Arc. Considering that the knowledge regarding sponges (Phylum Porifera) is still limited, in this study we attempt to estimate the richness of sponges of the order Poecilosclerida inhabiting the BB. We also compare these results with the richness recorded in adjacent regions of the Scotia Arc and neighboring areas (Tierra del Fuego, Malvinas, South Georgias, South Orkney and South Sandwich Is.). The studied material came from the Burdwood Bank, and it was collected during two research cruises in 2016 and 2017 onboard the OV “Puerto Deseado”. We also compiled previous records from the BB and other regions using the published literature. A checklist of 150 species of poecilosclerid sponges, 39 from the BB (with 22 of them representing new records and probably a couple of them new species for science), 49 from Malvinas Islands, 30 from Tierra del Fuego, 55 from South Georgias, 25 from the South Orkney, and 3 from the South Sandwich Islands. In total, 87% of the species were only recorded in one region. Only *Mycale* (*Aegogropila*) *magellanica*, *Iophon proximum*, *Isodictya setifera*, *Isodictya verrucosa*, *Myxilla* (*Myxilla*) *mollis*, *Tedania* (*Tedaniopsis*) *massa*, and *Tedania* (*Tedaniopsis*) *mucosa* (3.60%) were widely distributed among the studied regions. These preliminary results confirmed that BB sponge richness was previously underestimated. Until the present study, only 18 taxa were known from the BB, and we have doubled the known richness. Our results also suggest that the recorded richness may also constitute an artifact of the sampling effort exerted in each region. These results will be complemented with future studies considering the other orders and classes of the phylum Porifera.

### 3.4. Community Structure and Predation Marks on *Astarte longirostra* D’Orbigny (*Bivalvia*) from the Southwest Atlantic off Tierra del Fuego

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Astartids are a major component in marine ecosystems, playing important roles in trophic networks. At the southern Southwest Atlantic, *Astarte longirostra* lives partially buried in the sediment. Muricid and naticid gastropods feed on clams, drilling a hole in their valves. Drillholes made by predatory gastropods are useful tools for evolutionary and ecological studies, since they provide direct, preservable evidence of predation. The aim of this study was to analyse the community structure of and predation pressure on *A. longirostra* from the continental shelf off Tierra del Fuego (Southwest Atlantic), covering an area of 120 km<sup>2</sup>. Bottom samples were collected with a 13.94 dm<sup>2</sup> Van Veen grab during July 2014. Complete valves were separated from the sediment under a stereomicroscope. The valve length and drillhole diameter were measured with a micrometer eyepiece. *Astarte longirostra* valves occurred from 42 to 92 m. Predation pressure was assessed in 12 samples with at least 20 valves each, which were collected from 74 to 92 m, as clams were less frequent at shallower depths. The densities of this clam were variable (14–409 ind·m<sup>-2</sup>). Of the 4292 disarticulated valves analysed, 959 had drillhole marks. Due



to their irregular shape, 11.6% of the drillholes could not be attributed to predatory gastropods. The remaining 88.4% had rounded drillholes attributable to muricids and naticids, which exert strong predation pressure on clams. The predation rate was high (34.2% to 68.2%; pooled rate = 44.4%). Predation did not differ between left and right valves (Fisher's Test,  $p = 0.67$ ). The correlation between drillhole diameter and valve length was positive and highly significant, meaning that larger predators prefer larger clams. The size of the valves ranged from 0.62 to 5.85 mm. The size–frequency distribution was bimodal, with modes in 2–2.49 mm and 3.5–3.99 mm, suggesting the existence of two cohorts. Drillholes were significantly more frequent in the central sector of the valves. Of the seven species of predatory gastropods found, the naticid *Falsilunatia patagonica* was the most frequent.

### 3.5. Heterologous Expression and Characterization of *Cobetia amphilecti* KMM 296 Alkaline Phosphatase in *Nicotiana* spp.

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Alkaline phosphatase (ALP) of the PhoA family is an important enzyme in eukaryotes and certain gut and marine bacteria; it plays a crucial role in the detoxification of intestinal microbiome lipopolysaccharides (LPS) and excess of extracellular nucleotides, which overstimulate cell signaling and cause tissue inflammation in a host. Insufficient levels of this enzyme have been linked to several disorders in humans. This study aims at producing a recombinant PhoA (rec-PhoA) from the marine bacterium *C. amphilecti* KMM 296 in transiently transformed leaves of *N. benthamiana* and stably transformed *N. tabacum*, as well as elucidating its physical and chemical properties and anti-inflammatory potential. *Nicotiana* spp. have proven to be versatile and are extensively used as heterologous hosts in molecular farming. The *alp* gene was cloned into binary vectors pEff and pHREAC restricted with AscI, SmaI, and BsaI enzymes, respectively, and then incorporated into *N. benthamiana* leaves by means of agro-infiltration and into *N. tabacum* following the leaf disc method using the *Agrobacterium tumefaciens* EHA105 strain. The enzymatic activity of rec-PhoA from the transformed plants was confirmed in the leaves' lysate after purification using a metal-affinity column (Ni<sup>2+</sup>-IMAC-Sepharose) and, subsequently, Source-15Q ion-exchange sorbent (GE Healthcare, Buckinghamshire, UK). The purified rec-PhoA was visualized on SDS-PAGE as a 55 kDa band corresponding to the mature protein. The enzyme rec-PhoA was relatively thermostable and retained its activity at 15–45 °C up to 1 h, with the highest activity in Tris HCl (pH 9.0–11.0) at 35 °C, 40 min. It was salt-tolerant and divalent metal-dependent; Mn<sup>2+</sup> and Mg<sup>2+</sup> enhanced its activity. This study established that the plant-based expression of *C. amphilecti* PhoA in *Nicotiana* spp. is an appropriate method for the production of a pharma-grade LPS-free recombinant enzyme. This can be further explored for the cost-effective and massively scalable production of LPS-detoxifying ALP, for possible applications in human and animal therapy, as well as in plant growth promotion and biocontrol.

### 3.6. Importance of Studying Regional Biodiversity of Benthic Marine Diatoms and Cyanobacteria for Bioindication of Organic Pollution in Marine Environments

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Water quality monitoring using data on the biodiversity of living organisms has been employed for a long time. A saprobic system based on lists of organic pollution indicator species has been developed for freshwaters, but one has not yet been established for marine waters. Benthic diatoms and cyanobacteria are known bioindicators. The aim of this study was to assess the diversity of benthic diatoms and cyanobacteria in the epilithon of the three areas with different levels of eutrophication in Sevastopol Bay, Black Sea, for their use in general water quality assessment. The pollution of three areas of the bay was determined according to long-term hydrochemical data, as follows: Martynova Bay (MB)—relatively clean, Inkerman Bay (IB)—moderately polluted, Yuzhnaya Bay (YuB)—heavily polluted. The following indices of diversity were determined: Margalef (D), Shannon (H), Berger–Parker (IBP). The saprobic index was calculated according to Pantle and Buck's (S) method modified by Sládeček, using species significance indices (s) from the literature.

A total of 63 diatoms and 20 cyanobacteria species were found in Sevastopol Bay; among them, 21 and 6 were saprobionts, respectively. Based on diversity indices, it was found that the most favorable conditions for both diatoms ( $H = 2.65$ ;  $D = 7.67$ ;  $IBP = 0.28$ ) and cyanobacteria ( $H = 1.83$ ;  $D = 2.2$ ;  $IBP = 0.25$ ) were in MB. The worst conditions were formed in the YuB: for diatoms,  $H = 2.24$ ;  $D = 5.17$ ;  $IBP = 0.28$ ; for cyanobacteria,  $H = 1.04$ ;  $D = 0.67$ ;  $IBP = 0.5$ .

The diatom saprobic index showed that waters of MB are  $\beta$ -mesosaprobic:  $S_{MB} = 1.9$ . Meanwhile,  $S_{YuB} = 2.2$  ( $\beta$ - $\alpha$ -mesosaprobic) and  $S_{IB} = 2.5$  ( $\alpha$ -mesosaprobic). Therefore, the IB area is the most polluted, which is not in accordance with the obtained diversity indices and hydrochemical data. For cyanobacteria, the results of the estimations were also not consistent. Consequently, it is necessary to create a checklist of diatoms and cyanobacteria to calculate indices of indicator species with consideration of regional features.

### 3.7. Salinity Drives the Structure of Bryozoan and Entoproct Assemblage in Recreational Marinas of North Portuguese Coast

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Artificial structures such as marinas have increased in recent years due to the growing demand of recreational boating. Marinas modify the original environmental conditions and are thus considered a great stressor to native communities. Nevertheless, piles and floating pontoons provide new habitats that are prone to be colonized by fouling species. Bryozoans and entoprocts are common members of fouling communities that are frequently overlooked given their small size, particularly in the case of entoprocts. Furthermore, little is known about the influence of salinity on the assemblage structure of these two phyla. To improve this knowledge gap, as part of the project 'Marina-Forests' (<https://sciproj.ptcris.pt/175503PRJ>, accessed on 19 August 2024), fouling animals associated with floating pontoons were studied in 6 recreational (3 coastal and 3 estuarine) marinas of North Portugal. At each marina, 3 sites were haphazardly selected and the fouling community of 4 random  $10 \times 10$  cm grids per site was scrapped. The collected animals were washed through a 0.5 mm mesh sieve, fixed in a 4% formaldehyde solution, bryozoans and entoprocts were sorted and identified to the lowest level possible. The PERMANOVA

test was conducted to explore the effect of salinity on the assemblage structure of bryozoans and entoprocts and SIMPER routine to determine the species responsible for differences.

A total of 15 bryozoan species (621 colonies) and 7 entoproct species (1443 colonies) were found. The results showed that the assemblage structure of bryozoans and entoprocts was significantly different between coastal and estuarine marinas. The most important taxa responsible for differences were more abundant on coastal marinas except for the entoproct *Barentsia benedeni* and the bryozoans *Amathia imbricata* and *Conopeum seurati* that were more abundant in estuarine marinas.

The results of this study showed that salinity is an important driver of fouling assemblages. In view of many fouling species are invasive, our data also provided useful information to support decision-makers.

### 3.8. Evolutionary Functional Adaptations in the Gills of the Crab *Achelous hastatus* (Linnaeus, 1767) from Ganzirri Coast, Messina (Italy)

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Evolutionary events are primarily caused by transitions to physically distinct environments, such as shifting from sea to land. Changes in niches and ecological successions cause many speciation events. Significant morphological, physiological, reproductive, and behavioral adaptations are features of these evolutionary pathways. Among the Decapods, crabs (Brachyura) are marine in origin, but they have successfully colonized most intertidal and terrestrial habitats, developing significant functional adaptations. Our study aims to evaluate functional adaptations in the gills of the crab *Achelous hastatus* (Brachyura, Portunidae). We retrieved samples of 20 specimens coming from the coast of the Strait of Messina (location Ganzirri), Messina, Italy (38°15' N 15°36' E). Histologically, an additional outer layer surrounding the gill lamellae, composed of muco-protein material and scattered cells, many of them haemocytes, can be noted. Using confocal microscopy, a strong reactivity of this layer to both Toll-like receptor 2 and Na<sup>+</sup>/K<sup>+</sup> ATPase can be seen, suggesting that this layer is involved not only in the defense of the gills, which are in close contact with the external environment, but also in the exchange of ions to regulate salinity. These adaptations are consistent with the crab intertidal habitat, and similar adaptations have been found in the gills of brachiurians in mangrove forests in Brazil. Moreover, the presence of mucus may help in avoiding dehydration, and the reactivity to the antibodies tested suggests a reinforcement of the defense of the organism and ion regulation system. The data presented here provide new insights into the evolution and adaptations of marine organisms and contribute to our understanding of the internal defense and salt regulatory systems of this crab.

### 3.9. Fish Assemblages Reveal the Environmental Heterogeneity of Shallow Coastal Areas in Monte Hermoso, Argentina

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Within shallow coastal areas, the waters of open ocean dissipative sandy beaches can be differentiated into the surf zone, where wave action generates turbulent conditions, and the subtidal zone, characterized by higher depth and calmer conditions. These differences in hydrodynamic conditions could provide specific habitats for fish populations. This study conducted at Monte Hermoso beach, Argentina (38°59' S, 61°18' W), analyzed the spatial-temporal variation in fish assemblage diversity in the surf zone (SZ) and the adjacent

shallow subtidal zone (SSZ). Samples were simultaneously collected in both zones during low tide using a 10 mm (knot-to-knot) net, dragged for 300 m parallel to the shoreline. In the SZ, hand-towed trawls were conducted at a depth of 1.2 m, while in the SSZ, samples were collected at a depth of 5 m aboard a vessel at a constant speed of 4 km/h. Overall, 210 individuals belonging to 7 species were caught in the SZ and 1864 individuals belonging to 27 species in the SSZ. According to the graphical analyses based on the Olmstead–Tukey test (discriminant criterion = arithmetic means), *Odontesthes argentinensis* and *Ramnogaster arcuata* were dominant in the SZ, while *R. arcuata*, *Cynoscion guatucupa*, and *Anchoa marmorata* dominated in the SSZ. Richness and Shannon and Simpson's diversity, evaluated through Hill numbers, were higher in the SSZ during all seasons and showed no differences in winter. A high dissimilarity value was registered between the zones ( $\beta_{\text{SOR}} = 0.64$ ), and the nestedness component ( $\beta_{\text{NES}} = 0.50$ ) was higher than the turnover ( $\beta_{\text{SIM}} = 0.14$ ). This study represents the first record of shallow subtidal fish assemblages in Argentina and reveals that the wave action in the SZ negatively affects the richness and abundance of the fish assemblage. However, certain species, such as *R. arcuata*, exhibit remarkable plasticity in adapting to both environments.

### 3.10. The Influence of Depth on the Structure and Functioning of Benthic Communities on an Exposed Sandy Shore in the SW Atlantic

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Environmental characteristics such as depth, turbulence, and substrate composition influence the structure and dynamics of benthic communities in marine ecosystems. This study examines the biological descriptors ( $\alpha$ - and  $\beta$ -diversity, abundance, and biomass) and functional traits (mobility, feeding mechanism, and diet) of benthic communities across a depth gradient from the surf zone (SZ) to shallow subtidal zones (SSZs) on an exposed sandy shore of a small tourist beach (38°59' S, 61°18' W, Argentina). Samples were collected in spring 2018 and autumn 2019 from three depths (1 m, 5 m, and 14 m) using a benthic sledge (500  $\mu\text{m}$  mesh size). Overall, 10,055 individuals from 132 taxa were identified, with Arthropoda, Annelida, and Mollusca being the most represented groups. Arthropods were the most abundant at 1 m, while mollusks and annelids dominated at 5 m and 14 m, respectively. The maximum abundance and biomass were recorded at 14 m depth (1450.54 ind/100 m<sup>2</sup>, 264.72 g/100 m<sup>2</sup>), while  $\alpha$ -diversity was higher at 5 m depth ( $93 \pm 15.40$ ).  $\beta$ -diversity indicated high species turnover across the gradient ( $\beta_{\text{SOR}} = 0.72$ ;  $\beta_{\text{SIM}} = 0.55$ ), especially between 1 m and 14 m depths. More mobile, deposit-feeding, and detritivore organisms were found at 1 m, while sedentary, filter-feeding, and omnivore organisms were more abundant at 5 m and 14 m. The results indicate that biological descriptors and functional traits differed significantly across depths. At 1 m depth (SZ), the lowest abundance, richness, and diversity suggested that fewer species are adapted to this area, where higher mobility is advantageous to cope with the turbulence generated by continuous wave action. At 5 m and 14 m (SSZ), where environmental conditions are more stable, sedentary species prevail. Significant differences in diet could be related to the main food sources available in each zone. These findings suggest the existence of distinct benthic assemblages at variable depths. The heterogeneity of the marine benthic environment increases diversity and influences ecosystem functioning.

### 3.11. Inter-Annual Variability of the Macrobenthic Community in the Surf Zone of a Sandy Beach in the SW Atlantic

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The surf zone encompasses the region of sandy beaches where waves break along the shore. The benthic invertebrates inhabiting the water/bottom layer in this ecosystem represent a highly abundant and diverse community. Despite some efforts, more information is still needed on inter-annual variability. In this study, we integrate data from two periods, 2009 and 2015, to understand the variability in the biological descriptors of the community on a sandy beach in Argentina (39°00' S, 61°57' W) in the SW Atlantic. Samples (n = 3) were collected seasonally by hand-towing a benthic sled across transects parallel to the shore. A total of 62 species were collected; arthropods represented 55% and were mainly peracarid crustaceans. Differences in total density were registered between years ( $p < 0.05$ ), with ~39 ind/m<sup>2</sup> in 2009 (higher density in spring,  $p < 0.05$ ) and ~14,500 ind/m<sup>2</sup> in 2015 (higher density during autumn/winter,  $p < 0.05$ ). These differences were due to dense patches of two mysids, *Arthromysis magellanica* and *Neomysis americana*, registered in 2015 during the cold season, which can be explained by their migratory pattern, as they only arrive in the surf zone under certain physical conditions. Differences in species richness were found between years (15 in 2009 and 42 in 2015;  $p < 0.05$ ), with a high dissimilarity (>0.60%), given by both species turnover and nestedness. Changes in species richness were also detected between seasons: higher richness was registered in spring during 2009 ( $p < 0.05$ ), without differences between seasons during 2015 ( $p > 0.05$ ). The dissimilarity between seasons was also high (2009: >0.70%; 2015: >0.60%), mostly explained by the turnover component during both years. Given that the studied beach is subjected to low anthropogenic impact, the significant interannual variability observed in this ecosystem could be explained by its dynamic nature, emphasizing the importance of an extensive dataset to understand the physical and biological factors involved.

### 3.12. New Data on the Amphipod Fauna of the Hydrothermally Active Submarine Piip Volcano (Bering Sea)

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The submarine Piip Volcano (southwestern Bering Sea, 55°44' N, 167°26' E, 368–495 m) hosts the northernmost hydrothermal vents in the Pacific region. Benthic hydrothermal vent communities were explored during the two cruises of the RV *Akademik M.A. Lavrentyev* using the ROV *Comanche 18* (June 2016 and 2018). The samples were collected using the slurp-gun, net or mechanical arm of the ROV. Among the 131 species recorded on the volcano, there were at least 12 amphipod species (11 genera) from 10 families. Amphipod species were among the most common animals in the vent communities, some of which formed dense aggregations in bacterial mats. New data revealed that the three most abundant amphipod species are potentially new to science. Two closely related species of amphipods occurred exclusively on thick-layer bacterial mats, forming local aggregations, which was determined using a laser-scale up to 10,000 ind/m<sup>2</sup>. The morphology and phylogenetic analysis based on three molecular markers (CO1, 16S and 28S) suggest that these species belong to a new genus of the superfamily Lysianassoidea Dana, 1849. They differ from each other in their mouthparts (arming of Mx1), form of interantennal lobe, armament of gnathopods, uropods, form of epimeral plate 3, form of telson and other

characteristics, as well as by their CO1 and 16S markers. The third species, tube-building *Byblis* sp. (Ampeliscidae), was recorded on *Calyptogena* beds, bacterial mats and some sites without visible vent manifestations. These three amphipod species, which perhaps obligate for reducing habitats, increase the general number of obligate species of the volcano to eleven, belonging to six taxonomic groups (bivalves, gastropods, polychaetes, isopods, leptostracans and amphipods). The lysianassoid species possibly adapted to more pronounced reducing conditions than *Byblis* sp. Lysianassoid amphipods are diverse in Pacific hydrothermal vents and abundant in some of them. The relationships between amphipod faunas of the Piip Volcano and other hydrothermal vents of the northwestern Pacific are discussed. This research was supported by the Russian Science Foundation (grant 24-27-00242).

### 3.13. *Cephalopoda (Mollusca) from the Southwestern Austral Atlantic: An Ongoing Integrative Study*

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The Southwestern Austral Atlantic Ocean encompasses the southernmost tip of South America (the nearby shelf of Tierra del Fuego archipelago), the Burdwood Bank, the Malvinas/Falkland Islands, and their adjacent slope areas. Although it represents an area of great biological importance, knowledge on the marine biodiversity of this area is still limited. This is particularly noticeable in the case of cephalopod fauna species, many of which are only known from few (and occasional) records, and several nominal species have been mentioned without proper supporting information (photographs, vouchers, or similar). The aim of this research is to perform the first integrative study of the orders Octopoda and Sepiida of the Class Cephalopoda (Mollusca) occurring in the Southwestern Austral Atlantic, by combining morphological, morphometric, anatomical, and molecular characters. For that, newly obtained samples, collected between 2015 and 2018 by the Argentine research vessels GC-189 *Prefecto García* and BO *Puerto Deseado*, were studied. The specimens studied come from 37 sampling stations located between 53–55° S and 58–68° W in depths ranging from 40 to 785 m. Recently sampled cephalopods were photographed to document the coloration pattern of living specimens. Then, specimens were fixed for morphological, morphometric, and anatomical studies, which were performed in the laboratory; a small piece of tissue was preserved in ethanol for molecular studies (COI). For comparative purposes, the available type of material of all nominal species described from this area was studied. As part of this study, eight cephalopod species were recognized, comprising two species of sepiolids and six species of octopods. Octopods belonged to the genera *Enteroctopus*, *Muusoctopus*, *Graneledone*, and *Thaumeledone*, and, among the studied material, a new (undescribed) species was recognized.

### 3.14. *Citizen Science in the East Pacific Marine Realm: Bivalves Under Scrutiny*

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The growth of citizen science has revolutionized the knowledge of biological diversity worldwide, improving the knowledge on the species diversity for particular areas and improving the accuracy of known distributional ranges of the species. This study evaluates the current impact of the iNaturalist platform on understanding marine bivalve diversity along the East Pacific coast, from Alaska (71.39° N) to Cape Horn (54.86° S).

All marine bivalve observations along the East Pacific coast (to mid-June 2024) were obtained from the iNaturalist platform. The geographic coverage of these observations, and the accuracy of this set of information, was evaluated, and this information was contrasted with the total diversity currently known about in this area.

At the time of this analysis, a total of 77,207 marine bivalve observations, comprising 572 species and 62 families, were available in iNaturalist. This represents 45.0% of the total number of species and 77.5% of the total number of families known to the area. Most of these observations (80.5%) come from North America, followed by Central America (14.8%), and only 4.7% come from South America. The greater number of these observations (76.7%) appears to be identified at the species level, while the remaining observations refer to a higher taxonomic level (e.g., Genus, Family, or even Class). Observation quality was categorized as follows: 57.7% as “Research Grade”, 41.2% as “Needs ID”, and 1.1% as “Casual”.

Despite the great number of observations of marine bivalves currently available in iNaturalist, the coverage of this platform is still limited. This phenomenon is registered both at a geographic scale, particularly in the case of South America, and a diversity scale, with more than a half of the known species not yet documented therein.

### 3.15. Zooplankton in the Surf Zone and Adjacent Shallow Subtidal Waters: Differences in Community Patterns Between Close Seascapes

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Zooplankton that drift or weakly swim are influenced by water column structure and hydrodynamics. This investigation studied zooplankton communities in the surf zone (SZ) of sandy beaches, characterized by shallow and turbid waters due to wave propagation, and the adjacent shallow subtidal (SSZ), with calmer, deeper waters. This research presents a novel approach by concurrently studying these communities in both zones. Samples were collected bimonthly for a year at Monte Hermoso, Argentina (38°59' S, 61°18' W), using a 200 µm mesh zooplankton net by hand-trawl in the SZ and from a vessel in the SSZ. We analyzed species richness, Shannon and Simpson's diversity,  $\beta$ -diversity, taxonomic composition, and spatial variability in abundance between zones. No differences were found between zones in richness, but Shannon and Simpson's diversity were higher in the SZ.  $\beta$ -diversity reflected species turnover ( $\beta_{sim} = 0.294$ ,  $\beta_{nes} = 0.0982$ ), with approximately 40% taxonomic dissimilarity ( $\beta_{sor} = 0.392$ ) throughout the year. We identified 55 taxa in 15 major groups. Copepoda, Mollusca, Annelida, and Chordata accounted for over 90% of total zooplankton abundance. The results revealed abundance differences, with total abundances in SZ greater than SSZ ( $p < 0.001$ ; SZ = 2708 ind·m<sup>-3</sup>; SSZ = 134 ind·m<sup>-3</sup>), driven predominantly by copepods ( $p < 0.001$ ;  $r = 0.96$ ). These differences were reflected in copepod composition, with *Acartia tonsa* predominating in the SZ, while *Paracalanus parvus* dominated the SSZ. Both species exhibited similar seasonal patterns, peaking in warmer months for *A. tonsa* and in winter for *P. parvus*. In the SSZ, we registered more neritic taxa (e.g., Chaetognatha; Cnidaria, Ctenophora), whereas in the SZ we found more larval stages of benthic organisms from nearby populations (e.g., Echinodermata, Mollusca, Spionidae, Decapoda) and benthic organisms like Cumacea and Amphipoda. The coexistence of estuarine and neritic species highlights the interconnectedness of coastal habitats and provides insights for future ecological studies.

### 3.16. Assessing Distribution Patterns of Non-Indigenous Invertebrates in Ten Marinas Across Mainland Portugal, Madeira and Azores Through DNA Metabarcoding

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Non-indigenous species (NIS) pose significant threats to coastal ecosystems, causing ecological imbalances and biodiversity loss. Effective monitoring and management strategies are essential to mitigate these impacts, particularly in marinas and ports, which are crucial entry points for NIS due to shipping. This study assessed the spatial distribution of marine invertebrate fauna, particularly NIS, in 10 recreational marinas in Portugal (6 on the mainland and 2 each in Madeira and Azores) using DNA metabarcoding. Samples were collected from hard substrates, water and zooplankton in a single time-point survey in June and July 2020. Using the mitochondrial cytochrome c oxidase subunit I (COI) and the small subunit 18S rRNA gene (18S) markers, a total of 645 species, including 38 NIS, were detected. Only 5% of species and 5% of NIS were common to all marinas. The highest species richness was observed in the mainland North marinas, while Madeira exhibited the lowest richness. Ponta Delgada (Azores) recorded the highest number of NIS (15), whereas Madeira's marinas recorded the lowest (7 and 9). Species richness and taxonomic composition varied with sample type. A Principal Coordinate Analysis (PCoA) revealed three distinct groups: (1) North and Aveiro (mainland); (2) Lisbon (mainland); and (3) Madeira and the Azores. A PERMANOVA supported the theory that both sample type and marina significantly influenced species composition [ $P(\text{perm}) = 0.001$ ]. A comparison of the 38 NIS detected in this study, with the 2020 national NIS lists, revealed regional differences and spotted species that were not previously considered NIS but are now recognized as such: eight NIS in mainland Portugal, two in Madeira and two in Azores. This study provides valuable insights into the distribution of NIS and marine invertebrate fauna in Portuguese recreational marinas, while highlighting the efficacy of DNA metabarcoding for assessing spatial variability in invertebrate communities and supporting NIS surveillance programs.

### 3.17. Wind-Induced Changes in Zooplankton Diversity in a Temperate Sandy Beach Surf Zone: A Preliminary Methodology Using Neutral Red Staining

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Zooplankton responds quickly to environmental changes due to its sensitivity to hydrodynamic variations. This study investigates the impact of different wind events on zooplankton communities in the surf zone of a temperate sandy beach. Zooplankton



samples were collected before and after each wind event from May 2017 to July 2019 at Pehuen Co sandy beach. High-frequency wind speed data were used to categorize wind events by direction and duration. Physical and biological variables were analyzed using general Linear Models (LMs) and Generalized Linear Models (GLMs). Additionally, the samples were stained with neutral red to distinguish living organisms from dead ones. The study found that wind direction and duration significantly affect the zooplankton community. Short-duration wind events led to an increase in zooplankton abundance, with *Acartia tonsa* and *Paracalanus parvus* being predominant. Winds from the west were associated with the presence of inner continental shelf species such as *Ctenocalanus vanus* and *Euterpina acutifrons*, and to a lesser extent, *Calanoides carinatus* and *Labidocera fluviatilis*, along with typical surf zone copepods. In contrast, long-duration wind events significantly decreased zooplankton abundance, with SE–SW winds linked to adventitious taxa. In all the samples analyzed before/after the storms, mortality varied from 100% to 4% dead organisms after storms, with the highest mortality generally observed after short-duration wind events. Wind events, particularly their direction and duration, play a crucial role in shaping the abundance and composition of zooplankton in the surf zone of temperate sandy beaches. These findings highlight the importance of considering wind patterns in managing and conserving marine biodiversity. This research contributes to our understanding of how abiotic factors like wind influence coastal ecosystems. The results affect biodiversity conservation strategies in marine environments due to climate change.

### 3.18. Comparative Evaluation of the Feeding Habits of Sea Anemones (Cnidaria; Actiniaria)

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The behavioral patterns and feeding strategies of marine organisms represent significant aspects when comprehending the ecology, evolution, and dynamics of species. In addition, studies focusing on feeding habits in marine environments are fundamental for ecological conservation, since they are capable of revealing pollution levels. However, although individuals of the phylum Cnidaria have different life cycles and present a widespread distribution, studies of feeding behavior in anthozoans are scarce. Sea anemones (Cnidaria; Actiniaria) are predatory organisms and perform ecologically significant functions in benthic food webs. In this study, 42 individuals comprising three species, *Actinia bermudensis*, *Anemonia sargassensis*, and *Bunodosoma caissarum*, were collected in Florianópolis, Santa Catarina. The dissected organisms were analyzed internally using DNA metabarcoding techniques with amplification of the mtCOI sector in order to identify and compare their feeding patterns. The morphological analyses comprised an important stage in the partial identification of the fragments found in the gastrovascular cavity of the specimens, despite many of the food items being fragmented and half-digested. The sequencing method, in extension, revealed the identification of a diverse and extensive portion of predated organisms, illustrating the dietary variation in the species, mainly composed of arthropods, such as crustaceans and insects, annelids, and algae classified as ochrophytes.

### 3.19. Inter-Tidal Cirripede [Cirripedia: Thecostraca] Diversity in Goa: Three New Records

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Barnacles [Cirripedia: Thecostraca] are among the most biologically diverse and ecologically important marine crustaceans, commonly encountered in the inter-tidal and benthic habitats. Adult barnacles are sessile and can either be epibionts or foulers. The marine fauna of Goa, a coastal state on the west coast of India, is not well represented in general. Of the ca. 144 species of barnacles, distributed among 75 genera and 19 families,

reported to be present on the coasts of India, only five species from Goa have been identified so far.

The paucity of information and the dearth of data pertaining to the diversity and distribution of barnacles propelled the current study, which aimed at providing a preliminary account of barnacles along the Goan coast. Species diagnosis was mainly based on arthropodal characterization. A total of six cirripedes distributed among three families (Lepadidae, Chthamalidae, and Balanidae) and four genera are reported in the present study. Among these, three species constitute the first records from Goa. The only gooseneck barnacle recorded in the present study was found to be attached to the washed ashore debris on a sandy coastline. All acorn barnacles were recorded in rocky inter-tidal habitats, and a few individuals were found to colonize plastics lodged in rock crevices.

### 3.20. *The Morphological and Genetic Diversity of Species from the Genus Monocelis (Platyhelminthes: Proseriata) in the Littoral Zone of the White Sea*

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The taxonomy, anatomy, and biological features of free-living flatworms of the phylum Plathelminthes from the White Sea (Russia) have been studied poorly, and their diversity is greatly underestimated. The main obstacle to this is their challenging identification and the miniature sizes of considerable number of representatives of the phylum, microturbellarians' in particular. Microturbellarians of the genus *Monocelis* (Proseriata) are tremendously numerous in the littoral zone of the vicinity of the White Sea biological station of Moscow state university, and are represented, according to previous studies, by only two species—*M. lineata* (Müller, 1773) and *M. fusca* (Örsted, 1843). However, not all specimens examined during recent work match the characteristic features of the registered species.

Current research on *Monocelis* sp. specimens from the White Sea was carried out using an integrative taxonomic approach. We paid special attention to the significant variability of the worms' coloration. Based on the molecular phylogenetic analysis of the 18S gene and the 28S fragment sequences of the *Monocelis* sp. specimens, five clades were identified. These groups also were confirmed as candidate species by morphological features, including coloration, body proportions, structural features, and relative arrangements of the reproductive system parts. Also, we have obtained the complete sequence of the mitochondrial genome for one group for the development of universal primers.

The structure of the copulatory organ turned out to be the most important taxonomic feature: each identified group has a unique structure to the others. This feature gives us reason to assume the existence of reproductive isolation, which allows for the coexistence of several closely related species within one local biotope.

This work was carried out with the support of an RSF grant (No21-74-20028).

### 3.21. *The Unexpected Relationships Within Haliclystus (Cnidaria: Staurozoa)*

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Staurozoa is a unique group of stalked jellyfish, whose taxonomy has been significantly revised over the past two decades. However, phylogenetic relationships within the genera remain poorly understood, and the most numerous and problematic genus is *Haliclystus*, with 13 species, some of which have questionable validity. The current study aims to

investigate the diversity and phylogeny of *Haliclystus* species in the northern seas, using both morphological and molecular methods.

The specimens of *Haliclystus* were collected in the White, Barents, Okhotsk, and Japan Seas in 2022–2024. Their morphometric characteristics, rhopaloid shape and size, and cnidocysts arrangement were analyzed for morphological analysis. For the molecular analysis, COI, ITS, and 16S gene regions were sequenced.

As a result of morphological analysis, we identified two species with confidence: *Haliclystus auricula* (White Sea and Barents Sea) and *H. tenuis* (Sea of Japan). Three specimens from the Okhotsk Sea and the Sea of Japan have not been identified.

The resulting phylogenetic trees generally correspond to morphological identifications. Specimens of *H. auricula* from the White and Barents Seas form a single clade with species of *H. auricula* from the North Sea. The closest sister branch to the *H. auricula* clade is presented by the *H. antarcticus* species from the Southern Atlantic Ocean. Specimens of *H. tenuis* from the Sea of Japan are combined with specimens of *H. tenuis* collected in the North Sea and on the Pacific coast of Hokkaido. Unidentified samples from the Okhotsk and Japan Seas form two separate clusters that do not overlap with the available sequences from GenBank, and therefore may potentially be undescribed species. Our findings suggest two major groups, Atlantic-oriented and Pacific-oriented, with some specimens challenging this geographical classification. To establish phylogeographic relationships within the genus *Haliclystus*, it is necessary to extend studies using more samples.

### 3.22. A New Deep-Sea Phoronid Species from the Okhotsk Sea Basin

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Phoronids are a small type of exclusively marine invertebrate. They are distributed in most areas of the world ocean and often play an important role in marine communities as ecosystem engineers. However, the phylum consists of only 15 species, divided into two genera: *Phoronis* (11 species) and *Phoronopsis* (4 species). The identification of phoronids is usually very difficult due to the lack of distinctive morphological characteristics.

This contribution represents the first description of a deep-sea phoronid species. The material was collected in the Sea of Okhotsk from seven locations (50°58.434' N 148°14.182' E; 50°58.566' N 148°14.271' E; 53°59.946' N 146°14.868' E; 54°00.207' N 146°18.581' E; 50°30.930' N 155°18.456' E; 50°30.928' N 155°18.444' E; and 50°30.956' N 155°18.489' E). Samples were acquired from depths of 786 to 1551 m using a bottom sampler and a remotely operated underwater vehicle. The morphology and anatomy of the specimens were studied using light microscopy, histological sections, and three-dimensional reconstructions of metanephridia. The obtained data were used for cladistic analysis.

Based on the obtained morphological and anatomical data and cladistic analysis, it was concluded that the discovered specimens belong to a new species of phoronids from the genus *Phoronopsis*. The studied species differ from other species of this genus in the number of longitudinal muscle bands and the number of tentacles in the lophophore. In addition, spermatocysts, clusters of spermatozoa, have been described for the first time in phoronids. It has been suggested that spermatocysts increase the probability of sperm delivery from one individual to another and, thus, are an adaptation to a deep sea lifestyle.

### 3.23. Chemodiversity and Bioactive Natural Products from Deep-Sea-Sourced *Streptomyces* (*Actinomycetota*, *Kitasatosporales*, *Streptomycetaceae*) Strains

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Marine environments harbour a wealth of diverse and underexplored microbiota, including species from the actinobacterial genus *Streptomyces* (Actinomycetota)—remarkably prolific producers of a wide array of natural products with unique bioactivities and high potential for drug discovery and other applications.

In our study, we explored liquid culture supernatants from various *Streptomyces* strains sourced from deep-sea environments, corresponding to different species, aiming to uncover their chemical diversity and potentially identify valuable natural products. To achieve this, we followed a multi-step extraction procedure involving liquid–liquid and sorbent-assisted extraction steps. Leveraging a high-throughput ultra-high-performance liquid chromatography–electrospray ionisation–high-resolution mass spectrometry (UPLC–ESI–HRMS) dereplication workflow, we employed molecular networking (MN) and cheminformatic approaches to obtain insights from complex spectral datasets. Our dual focus was to identify known compounds (chemical dereplication) and highlight potentially novel ones within the extracts’ “chemical spaces”. We further fractionated the extracts, performed general and targeted thin-layer chromatography (TLC) assays, and purified the fractions for structural elucidation using UPLC–ESI–HRMS and nuclear magnetic resonance (NMR) spectroscopy, guided by the dereplication.

The investigated strains of marine-derived *Streptomyces* displayed diverse and intriguing chemical profiles both within and between species. The primary dereplicated compounds included linear and cyclic hydroxamate siderophores from the “ferrioxamine family” and autoregulatory inhibitors of spore germination from the “germicidin family”. Additionally, other natural products, such as carboxylic acid derivatives, dipeptides, and nucleosides, were annotated, all of which with significant application potential.

The described analytical workflow fortified by state-of-the-art cheminformatic approaches allowed us to gain insights into the chemical spaces of the extracts and distinguish potential compounds of interest. The data from MNs are crucial for guided purification during extract fractionation, complementing classical techniques for efficient compound identification and, ultimately, NMR structural elucidation.

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### 3.24. A Comparative Analysis of Two Phoronid Species: *Phoronopsis viridis* and *Phoronopsis harmeri* and the Problem of Phoronid Taxonomy

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Phoronids are a small phylum of marine invertebrates consisting of only 15 species, most of which have a worldwide distribution. In the majority of marine ecosystems, phoronids are the dominant species, forming high-density aggregations and thus playing an important edificatory role.

The external morphology of phoronids is poor, and for an exact identification of the species it is necessary to obtain a complete series of histological sections and construct a three-dimensional reconstruction of some internal of its organs. If such morphological work is carried out, it becomes clear that most phoronid samples from previously unexplored water areas are new species. Another problem in phoronid taxonomy is the synonymization of species, which in many cases is very controversial. One important issue in phoronid taxonomy is the description of species by their larval stages—actinotrochs. More than 50 so-called “larval species” have been described, which indicates the existence of undescribed species of adult phoronids.

The present work is devoted to the anatomy and phylogenetic analysis of two populations of *Phoronopsis harmeri*: from Vostok Bay, the Sea of Japan, and from Friday Harbor, on the Pacific coast of North America. Morphological studies made it possible to describe

in detail the structure of organ systems important for species identification, such as the lophophore's shape, the structure and number of longitudinal muscle bands, the structure of giant nerve fibers, and the shape of metanephridia. For molecular genetic analysis, we studied the sequences of the 28S rRNA and COX1 genes obtained by us from *Phoronopsis harmeri/viridis* samples from the indicated locations, as well as those taken from the Gen-Bank and belonging to individuals of these two species from different waters of the World Ocean. The obtained data allow us to raise the question of the possible revalidation of the species *Phoronopsis viridis*.

### 3.25. A Ground Approach in Studies on the Diversity of Clupeiform Fishes (Order: Clupeiformes) on the Malabar Coast of Kerala

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Clupeiform fishes are represented by a high diversity of species among commercial marine landings on the Kerala coast. They are a globally distributed clade with nearly 400 marine, freshwater, and diadromous species that form large schools and occupy a diverse array of trophic guilds and habitats. However, during this ground-level approach to exploring clupeid fishes on the Malabar Coast, thirteen species belonging to three families were recovered. Among the clupeid fishes recovered during the study period, members of the family Engraulidae were most abundant, followed by Pristigasteridae and Clupeidae. Meanwhile, with respect to the population size, engraulids and clupeids were more abundant. Regarding oil sardine, *Sardinella longiceps* is the most abundant clupeiform fish along the Kerala coast, and it supports the state's economy through a single-species fishery. Out of the 112 fish collected during the study period, 83 were found to be members of the order Clupeiformes, belonging to three families: Engraulidae, Clupeidae and Pristigasteridae. Six species of Engraulidae, four species of Pristigasteridae and three species of Clupeidae were identified, but the population size of Engraulidae (48%) was highest, followed by Clupeidae (40%) and Pristigasteridae (12%). An examination of ectoparasitic infestation among these fishes was also thoroughly studied. Two parasitic crustaceans belonging to the two groups Isopoda and Copepoda were found to be infesting the body surface, buccal cavity, opercular cavity, and gills of the clupeid fishes.

## 4. Microbial Diversity and Culture Collections

### 4.1. Diversity of Enterococcus Strains in Raw Donkey Milk: Evaluating Their Technological Attributes and Safety Profiles

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Raw donkey milk, a lesser-known but nutritionally valuable resource, has gained recognition for its unique composition and potential health benefits. It is within this context that the diversity, technological attributes, safety considerations, and probiotic potential of enterococci from raw donkey milk are explored. This study aimed to determine whether these isolates could be potential probiotics in donkey milk. A total of 25 Gram-positive, catalase-negative bacteria were obtained from twenty raw milk samples collected from a donkey species from Southern-Western Haryana, utilizing selective microbiological media. All isolates underwent identification through both phenotypic and molecular techniques and were subjected to a comprehensive assessment of their technological and probiotic properties. To assess their viability as starter or adjunct cultures, the isolates

were evaluated for their susceptibility to antibiotics, absence of virulence factors, and absence of hemolytic activity, and the safety analysis of these isolates revealed that the majority exhibited susceptibility to clinically important antibiotics, such as vancomycin. Overall, the findings of this study suggest that the Enterococci strains that are present in raw donkey milk are unlikely to pose a significant risk to human health. Additionally, they display noteworthy technological characteristics such as high antibacterial efficacy against *Staphylococcus aureus*, *Pseudomonas*, *Escherichia coli*, etc. It was possible to enhance the antibacterial activities against these pathogenic strains by using various induction techniques and use them as potential probiotics in fermented food products.

#### 4.2. Gut Microbiome Diversity in *Apis mellifera*: Insights from Culture-Dependent and Culture-Independent Methods

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**Introduction:** The intricate and ever-changing gut microbiota of *Apis mellifera* bees is an essential component of their overall health and welfare. A wide variety of probiotic bacteria make up this microbiome, which helps with immunity, aids with digestion, and guards against infections. Various factors, like nutrition, ambient conditions, and interactions with other microbes, determine the diversification of these gut bacteria.

**Methods:** A combination of culture-dependent and culture-independent methodologies are used to study the biodiversity of probiotic bacteria in the gut of honey bees. Culture-dependent techniques entail separating bacteria on certain media, but culture-independent techniques like 16S rRNA gene sequencing offer a thorough picture of bacterial communities. In order to extract DNA for sequencing, worker bee stomach samples are collected and processed. Sequencing data is analysed, bacterial species are identified, and relative abundances are evaluated using bioinformatic methods.

**Results:** This study revealed that a few key bacterial species, such as *Lactobacillus* and *Bifidobacterium*, dominate the gut microbiome of honey bees. These microbes are necessary for the production of short-chain fatty acids, vitamins, and the fermentation of pollen. These groups are highly diverse, with several strains displaying unique functional skills. Disparities in gut bacterial populations are also influenced by hive location and seasonal fluctuations.

**Conclusions:** Maintaining a diverse and balanced gut microbiome helps bees withstand environmental stresses and enhances their immunity against diseases. Future research should focus on understanding the specific roles of different bacterial strains and developing strategies to support and enhance the gut microbiome through probiotic supplements modifications. This could aid in improving bee health and sustaining their populations, which are vital for global pollination and agricultural productivity.

#### 4.3. Synthesis, Digitization and Bioprospecting of Culture Collections

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The National College Culture Collection Centre (NCCCC) and Kristu Jayanti Culture Collection Centre (KJCCC) represent pivotal repositories of microbial diversity, collectively housing over 300 species of microorganisms. These culture collections serve as essential resources for biotechnological research, facilitating bioprospecting endeavors aimed at uncovering novel applications in various fields. One of the primary focuses of research at these centers is bioprospecting, which involves screening microbial species for their potential to produce valuable compounds such as organic acids, enzymes, and antibiotics. This exploration capitalizes on the metabolic diversity inherent in microbial communities, aiming to identify bioactive substances that can be utilized in pharmaceuticals, agriculture, and industrial processes. The NCCCC and KJCCC play critical roles in biodiversity conservation and sustainable development by preserving and studying microorganisms from

diverse ecological niches. Through systematic screening and characterization, researchers at these centers seek to harness the biochemical capabilities of microorganisms for societal benefit while promoting environmental stewardship. Furthermore, these culture collections contribute significantly to advancing scientific knowledge and technological innovation. By collaborating with academia, industry, and governmental agencies, the centers foster interdisciplinary research that addresses pressing global challenges, such as antibiotic resistance and environmental degradation. The NCCCC and KJCCC exemplify the importance of microbial culture collections in biotechnological research and development. Their efforts in bioprospecting for organic acids, enzymes, and antibiotics underscore their commitment to exploring the vast potential of microbial biodiversity for sustainable solutions and industrial applications.

#### *4.4. Honey Is a Natural Postbiotic Product and Source for Beneficial Microorganisms*

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Honey's antioxidant effects stem from its phenolic constituents, which protect human cells and the bloodstream upon consumption. The antioxidant capacity of honey varies based on its floral source, with darker-colored honey possessing greater antioxidant activity due to its higher phenolic content. These phenolic components are also linked to honey's anti-inflammatory qualities, as honey can interrupt inflammatory mediators and decrease pro-inflammatory cytokine levels. Consequently, the anti-inflammatory and antioxidant activities of honey are intrinsically connected. Certain honeys are used in treating wounds, burns, and ulcers due to their anti-inflammatory, antioxidant, antibacterial, and wound-healing properties. However, the systemic effects of honey when consumed orally remain understudied. Honey, a primary product of beekeeping has been consumed by humans since ancient times. Plant nectar, produced by glands in flowers, is necessary for honey production. The productive microbiota of honey bees and their enzyme, invertase, convert nectar into honey through enzymatic processes. The fermentation process turns honey into a postbiotic substance. Bees stimulate the enzyme glucose oxidase, converting glucose into gluconic acid and hydrogen peroxide, which are crucial for honey's taste and biological functions. Given these characteristics, honey should be recognized as a natural metabiotic (postbiotic) substance for treating various health conditions. Funding: The study is supported by the IDB RAS Government's basic research program in 2024 (No. 0088-2024-0009).

#### *4.5. Cemetery Soil as a Potential Factor in the Spread of Antibiotic Resistance*

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Antibiotic resistance has increased significantly over time. Key factors contributing to this problem include the improper use of antibiotics in medical settings, the misuse of antimicrobials in animal farming, and the presence of antibiotics in the environment, such as through sewage transmission and cemetery soil, which increases the risk of further transmission.

Graveyard soil is known to be contaminated with heavy metals and other hazardous elements, but little is known about its effect on antibiotic resistance. Cemeteries may play a key role in the spread of antibiotic resistance due to limited rules governing burial practices and graveyard management, particularly in areas that are prone to microbial incursion into the environment, such as lakes. Only a few studies have been carried out on this topic, but they all corroborate the presence of drug-resistant bacteria in graveyard soil. Given bacteria's potential to transfer antibiotic resistance, these findings are concerning and require further investigation.

My PhD project examines the significance of graveyard soil as a potential reservoir for antibiotic resistance. Soil samples were collected from a few cemeteries in the Kuyavian-

Pomeranian voivodeship near water reservoirs, including on the surface and beneath coffins during exhumations. The samples were examined for the presence of micro-organisms that are resistant to certain antibiotics (amoxicillin, cefuroxime, doxycycline, and tetracycline), as well as genes linked with resistance to  $\beta$ -lactams and tetracyclines and sulfonamides.

Variations in resistant colony counts were discovered based on the sampling depth, and resistance genes were also identified, demonstrating that cemeteries may actually function as sources of antibiotic-resistant bacteria and their genes.

Inappropriate cemetery placement, such as being located on the hills or near water bodies with high groundwater levels, may hasten the transmission of antibiotic-resistant bacteria and their genes from graveyards to the surrounding natural environment. More research is needed to properly understand the function of cemeteries in spreading antibiotic resistance.

#### 4.6. Exploring the Presence of Human-Related Microsporidian Spores in Lizards from Bombali District, Sierra Leone

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Our investigation into the presence of human-related microsporidia in lizard feces is not only a scientific endeavour, but a crucial study with potential implications for public health. While these species have been found in various vertebrates, their zoonotic potential remains poorly understood. Thus, previous studies have detected spores related to *Encephalitozoon* spp. in lizards' feces from Baltimore Zoo (the US), specifically in the lizard *Shinisaurus crocodilurus*. Although they did not develop systemic infections, the presence of spores of *Encephalitozoon* may represent a source of infection. To determine the presence of opportunistic microsporidia species (*Enterocytozoon bieneusi* and *Encephalitozoon* spp. [*E. intestinalis*, *E. hellem*, and *E. cuniculi*]) in lizard feces from different urban/rural areas in Bombali District, Sierra Leone, we undertook a rigorous process of investigation. DNA was extracted from nineteen lizard fecal droppings collected in 2019 from different locations, including built-up areas in Makeni city and a rural/leisure area in the village of Mankneh, using the Fast-Prep for Soil<sup>®</sup> kit. A SYBR Green real-time PCR revealed that none of the samples monitored were positive for any of the four human-related microsporidia species studied. Although this finding implies a low risk for humans from direct/indirect exposure to microsporidian spores that are potentially present in lizards' feces, the carrier state and potential environmental contamination, as well as human exposure, cannot be excluded. Owing to the fact that lizards are very common in Sierra Leone, such as the species of *Tarentola parvicarinata* (a wall gecko), we recommend further molecular analysis for the detection of other important microsporidian species that have previously been detected in reptiles, such as *Pleistophora* spp., which could also affect humans. The need for further studies is also highlighted by the fact that we have reported some circulation of these microsporidia species in wild and domestic animals in Sierra Leone.



#### 4.7. Wildlife as Key Reservoirs of *Staphylococcus aureus* Genetic Variants

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*S. aureus* is a versatile pathogen capable of colonizing various hosts. Studying *S. aureus* in wildlife provides crucial insights into its ecology and evolution. Understanding the genetic diversity and distribution of these strains is essential for comprehending transmission dynamics, potential zoonotic risks, and the overall impact of wildlife reservoirs on public health and animal populations. This study compares different clonal lineages of *S. aureus* in wild animals, including owls, hares, rodents, and hedgehogs.

Mouth and rectal swab samples were collected from 204 wild rodents, 114 wild owls, 83 wild hares, and 110 wild hedgehogs. The samples were incubated in Brain Heart Infusion broth with 6.5% NaCl for 24 h at 37 °C. Subsequently, the inoculum was seeded onto Baird-Parker agar and CHROMagar MRSA. Multilocus Sequence Typing (MLST) and *spa*-typing were performed to investigate the genetic diversity of *S. aureus* isolates.

Three *mecA*-MRSA isolates were identified from hares, three from rodents, and two from hedgehogs. Additionally, one *mecC*-MRSA was isolated from owls, three from rodents, and three from hedgehogs. For MSSA, 22 isolates were found in owls, 28 in rodents, and 17 in hedgehogs. Regarding MLST and *spa*-typing, two *mecC* isolates from hedgehogs were ST130 (CC130) and *spa*-type t843, while another was ST49. The three *mecC* isolates from rodents were ST1945, and the owl isolate was ST1245; all belonged to CC130 and *spa*-types t1535 and t843. Most *mecA*-MRSA isolates were associated with human-related clonal lineages like CC8 (hedgehogs) and CC22 (rodents). The isolates from hares belonged to clonal lineages associated with rabbits for consumption (ST2855-t1190). The MSSA isolates showed high clonal diversity, including over 30 STs and 40 different *spa*-types.

These findings highlight the significant clonal diversity of *S. aureus* in wild animals, underscoring the importance of wildlife as reservoirs for various lineages, with implications for both animal and public health.

#### 4.8. Exploring the Interplay of the Semiovaginal Microbiome

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The microbial communities inhabiting the reproductive tract play an important role in reproductive health. During unprotected sexual intercourse, the microorganisms present in the semen and vagina of heterosexual couples interact closely with each other. The concept of the “semiovaginal microbiome” has recently emerged to refer to all microorganisms

present in both environments. Each partner influences the microbial composition of the other, even sharing several bacterial taxa. Therefore, to improve the understanding of this interaction, we aimed to analyse the microbial composition of semen and vagina samples from heterosexual couples and to assess their similarity in terms of composition and diversity. The selected cohort consisted of 50 infertile couples before entering infertility treatment from whom semen and vaginal samples were collected. Bacterial genomic material was extracted from the samples, followed by amplification and sequencing of the V4 hypervariable region of the 16S ribosomal RNA (rRNA) gene to allow for the taxonomic assignment of the microorganisms at the genus level. A total of 443 bacterial genera were identified in the semen samples and 107 genera in the vaginal samples.  $\alpha$ -diversity analysis revealed that the semen samples had higher bacterial diversity and richness than the vaginal samples. In addition,  $\beta$ -diversity analysis showed significant dissimilarities in bacterial genus composition between the semen and vaginal samples of the couples. However, 39 bacterial genera were shared by both niches, supporting an active exchange of microorganisms during unprotected sexual intercourse. Focusing research on couples' microbiomes from a holistic view may provide a better understanding of their interactions, which could help to develop more effective strategies to improve reproductive success.

#### 4.9. Evaluating Glomalin Variability in the Semi-Arid Region of Caldenal: Effects of Seasonality and Land Use

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Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that form close associations with the roots of host plants, extending their root system and affecting plant physiology and communities. The extraradical hyphae of AMF produce glomalin, an adhesive and stable glycoprotein that is extracted from the soil in fractions such as TG (total glomalin) or EEG (easily extractable glomalin). Since the extraction process of glomalin also extracts other types of compounds, the term GRSP (glomalin-related soil protein) is used. When GRSP is quantified using the Bradford method, it is referred to as BRSP (Bradford-reactive soil protein). In the semi-arid subregion of Caldenal, Argentina, the conversion of native forests to agricultural grazing plots has been significant, with few studies on soil microbiology. This study evaluated the influence of seasonality and land use on BRSP concentration. The selected land uses were native forest (dominated by *Neltuma caldenia*), alfalfa (*Medicago sativa*), weeping lovegrass (*Eragrostis curvula*), and soybean (*Glycine max*). The analyses showed no significant differences in BRSP concentration between seasons (winter vs. summer). However, there were significant differences in BRSP concentrations across different land uses during the summer. Soybean plots had the lowest BRSP concentration, with significant differences compared to weeping lovegrass and forest, with the forest showing the highest BRSP values. Additionally, no significant differences were found between alfalfa and the other land uses. The main factors influencing BRSP concentration are vegetation diversity, intensity of agricultural activity, and differences in AMF diversity,

as different AMF taxa vary in their allocation of resources to glomalin production. The results suggest that land use change affects BRSP concentration in the Caldenal subregion, highlighting the importance of this protein in soil stability and erosion prevention.

#### 4.10. Distribution of Fungi of the Genus *Alternaria* in Black Point-Infected Wheat Seeds in the Czech Republic

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*Alternaria* are widespread fungi, saprophytes, and pathogens of agricultural plants which often colonize the seeds of cereal crops and are part of the complex of pathogens that causes black point. Their seeds and products are capable of synthesizing toxic metabolites, mycotoxins, which have an impact on the health of humans and animals.

This study was carried out at the Crop Research Institute in Prague, Czech Republic. Winter wheat seeds of the 2021–2022 harvest of the most common varieties were collected in different regions of the country. Mycological analysis of the seeds was carried out in the laboratory of Genetics and breeding methods of the Crop Research Institute. The identification of fungi of the genus *Alternaria* was carried out based on the morphological and cultural characteristics of colonies on PCA, the size and structure of conidia, and sporulation habits.

As a result of this study, the distribution of fungi of the genus *Alternaria* in winter wheat seeds affected by black point was studied. It was found that the frequency of occurrence of fungi of the genus *Alternaria* in the mycoflora of affected winter wheat grain in the Czech Republic in 2021 was 64.6%, and in 2022, it was 74.3% on average. The most frequently identified species were those belonging to the section *Alternata*, including *Alternaria arborescens*, *Alternaria alternata*, and *Alternaria tenuissima*. Their share in the population was 85.8% in 2021 and 96.5% in 2022. The species *A. arborescens* occupied a dominant position (47–49%) among fungi of this genus in the *Alternaria* population in the Czech Republic.

The *Alternaria* population in wheat seeds in the Czech Republic is dominated by fungi belonging to the section *Alternata*, which are capable of toxin formation. Regular monitoring of species diversity is necessary given its potential harmfulness when using wheat seed products.

#### 4.11. Inter- and Intraspecies Variability Among Yeasts Isolated from Dairy Products

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**Introduction:** Yeasts are usually considered the spoiling agents of food products, although they can be used in many fermentation processes, in particular in milk products. As lactic acid bacteria, they contribute some beneficial properties affecting the organoleptic features of dairy products and acting as probiotics. Studying the variability of yeasts isolated from dairy products can broaden our understanding of yeast genome organization and functioning and define some genetic markers to differentiate their species and strains. The purpose of the present study was to examine inter- and intraspecies variability among yeast species isolated from Ukrainian dairy products with the help of several molecular genetic markers.

**Methods:** Species of *Saccharomyces*, *Kluyveromyces* and *Rhodotorula* genera isolated from dairy products were used in this study. Bioinformatic analysis was carried out on nucleotide sequences of 18S rRNA, 26S rRNA and ITS-sequence from GenBank using

the FaBox (1.41), MEGA 10 and Geneious R6 programs. Molecular genetic analysis was performed by ISSR PCR and RT-qPCR.

**Results:** The results of the bioinformatic analysis showed a strict differentiation between *Ascomycota* and *Basidiomycota* species using conservative sequences of rRNA genes and ITS-sequences. Molecular genetic analysis using ISSR markers revealed a high level of inter- and intraspecies variability of yeast species. The size of the amplification products varied from 300 to 3000 bp; the number of amplicons in the total spectrum for each species was 16–33; and the percentage of intraspecies polymorphisms was 68–100%. A high level of variability in the expression of flocculin genes, which could be associated with different adhesive properties, was also detected between the various species and strains.

**Conclusions:** The results obtained in this study showed that the highest level of polymorphic loci was observed between *K. marxianus* strains using ISSR markers. Relevant and informative markers were suggested to analyze the inter- and intraspecies diversity of yeast isolated from dairy products.

## 5. Phylogeny and Evolution

### 5.1. First Karyotypic Description of *Echimys chrysurus* Zimmermann, 1780 (Rodentia, Echimyidae, Echimyinae) from the Amazon and Chromosomal Patterns Among Echimyidae Lineages

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The Echimyidae family is the largest group of Hystricognathi rodents, with 28 genera and 103 species, which are widespread throughout South and Central America. Despite a remarkable degree of karyotypic diversity, with a diploid number (2n) ranging from 14 to 118 and autosomal fundamental number (FNa) ranging from 14 to 168, the karyotypes of eight genera are not known. Here, we determine the karyotype of *Echimys chrysurus* species from the eastern Amazon, based on two samples collected in Paragominas and Santa Bárbara municipalities, Pará state, Brazil. The karyotype was analysed through G-banding, C-banding, and Fluorescence In Situ Hybridization (FISH) with telomeric and 18S rDNA probes. *Echimys chrysurus* has a 2n = 80/Fna = 134 karyotype. The autosomal set consists of 28 meta/submetacentric pairs (1–28) and 11 acrocentric pairs (29–39); the X chromosome is large and acrocentric, and the Y chromosome is small and acrocentric. The constitutive heterochromatin is distributed in small amounts at the centromeric region of all autosomes and the X- and Y-chromosomes. FISH with telomeric probes showed only distal signals, and FISH with rDNA 18S probes exhibited a signal in the interstitial region of a small metacentric pair. *Echimys chrysurus* is nested within a cluster with 13 other Echimyini (Echimyinae) representatives, in which about 89% of the karyotypes show a tendency towards high 2n (50–118) and an elevated number of banded chromosomes (Fna = 100–68). Similar to the other three subfamilies, the Echimyinae have karyotypes exhibiting high and low 2n (from 14 to 118) but with distinct 2n ranges: Capromyinae (34–88), Carterodontinae (66), and Euryzygomatomyinae (32–65). The majority of Echimyidae taxa exhibit distinct karyotypes for each species but with a prevalence of banded chromosomes, regardless of the 2n value. This indicates that the chromosomal evolution of this family was complex and with intense genomic reshuffling due to several rearrangements, such as fusion/fission, pericentric inversions, and multiple translocations.

### 5.2. Chromosomal Rearrangements Drive Diversity in Tree Rats of the Genus *Oecomys* (Rodentia, Sigmodontinae, Oryzomyini)

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*Oecomys* is a taxonomically complex and cytogenetically diverse genus with a controversial intraspecific phylogenetic relationship. Karyotypic analyses, using whole chromosome probes from *Hylaeamys megacephalus* (HME, Sigmodontinae, Oryzomyini) in some taxonomic lineages of this genus have been detecting the rearrangements that shaped their karyotypes, in addition to revealing relevant insights into the taxonomic status of these taxa. Thus, intending to investigate the chromosomal evolution of the genus, we characterized the karyotype of *Oecomys rutilus* (ORU) with HME probes, establishing chromosomal homology maps with the karyotypes of other congeners that already mapped with the same set of probes. The chromosomal phylogeny obtained by Maximum Parsimony analysis recovered the genus *Oecomys* as monophyletic, with moderate bootstrap support (68%). This clade branches into two large groups, the first including *O. rutilus* followed by *O. catherinae*—Rio de Janeiro and *O. catherinae*—Pará; the other group includes *O. auyantepui* followed by *O. paricola* cytotype C and *O. paricola* cytotype A + cytotype B. We detected that these taxa underwent intensive reorganization in their karyotypes, and the rearrangements that produced this diversity were 15 pericentric inversions, 12 centric fusions, 11 fissions, 5 *in tandem* fusions, 8 simple translocations, 1 neo-centromere and addition/deletion of constitutive heterochromatin on two autosomes and the X chromosomes. However, despite the high number of chromosomal rearrangements found, we identified some entirely conserved syntenic blocks shared among all species analyzed. From these data, we hypothesized a putative ancestral karyotype ( $2n = 60/FN = 62$ ) for the species analyzed. We also detected exclusive characteristics for ORU, the syntenic blocks HME 1/20/4, HME (16,17)/3, HME 2/1, HME 2/7, HME 5/(9,10)/8, HME 19/12. Here, we provide an overview of the chromosomal reorganization of the genus that points to a high chromosomal diversity and demonstrates that chromosomal rearrangements play a significant role in the radiation of these species.

## 6. Plant Diversity

### 6.1. Predictive Models of the Species Diversity and Biomass of the Herb Layer During Changes in the Stands of Dark Coniferous Forests

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**Introduction:** The study of forest biodiversity, productivity and dynamics is one of the priority areas of forest ecology and forest science in many countries. At the same time, the understorey layers are still insufficiently studied. The aim of this work is to study the interactions between the stand and the herb layer in order to develop predictive graphical models.

**Methods:** Genetic forest typology [17] and generally accepted methods of studying forest vegetation [18] are chosen as the methodological basis. We have studied the most widespread and productive forest type in the South Urals (Russia): moss spruce forest. Primary dark coniferous forests and secondary birch and aspen forests of different compositions and ages were studied.

**Results:** The studies carried out confirmed our null hypothesis regarding the possibility of modelling the species diversity and biomass of the herb layer according to the structure and age of the stand. All the criteria studied: the species richness of the plant community, the biomass of the herb layer, as well as the  $\alpha$ -diversity estimated on the basis of diversity indices, showed a high sensitivity to the age and composition of the stand.

**Conclusions:** The models we have developed can be combined with remote sensing data to provide large-scale data on difficult-to-measure but important characteristics: the biodiversity and biomass of the herb layer.

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## 6.2. Mushrooms Biodiversity Inventories of the Trara Mountains in Northwest Algeria

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Wild mushrooms are a valuable source of natural non-timber forest products, as well as a promising source of bioactive biomolecules with ecological utility in the proper functioning of ecosystems. The aim of this work is to acquire a knowledge base on the diversity of higher fungi in their natural biotopes, and to understand their ecology and identification in order to make a contribution to their inventory in the study area. The Trara massif is a coastal mountain range in the western extension of the Tellian Atlas, located on the north-western coast of Algeria. The massif's vegetation consists mainly of pines and cypresses. The Mediterranean climate is characterized by seasonal rainfall and a dry period. Few data are available on macromycetes in Algeria, and in this context we thought it would be useful to carry out a preliminary inventory of fungi in this region. The mycological surveys were carried out from 2021 to 2023. Recognition of harvested macromycetes was based on a series of apparent macroscopic characteristics and microscopic observation in the fresh state or with Lugol's and Melzer's reagents, and measurement of spores and fungal structures. Macrochemical reactions of the various parts can also be useful. The nomenclature adopted in this research is that of the Index Fungorum. This survey revealed 36 species belonging to 17 families classified into 4 orders with 26 genera: Boletaceae, Suillaceae, Paxillaceae, Tricholomataceae, Pleurotaceae, Hygrophoraceae, Mycenaceae, Marasmiaceae, Psathyrellaceae, Agaricaceae, Amanitaceae, Lycoperdaceae, Strophariaceae, Hymenogastreae, Pluteaceae, Gloeophyllaceae, and Pezizaceae. Three species of Lichens and one Myxomycete were also inventoried. Some species were good edibles, such as *Agaricus campestris*, *Pleurotus eryngii* var *ferulae*, and *Lepista nuda*. Access to trustworthy information on the state and location of ecological communities makes biodiversity conservation and sustainable management in forested landscapes much easier.

### 6.3. Temporal Patterns of Insect Pollinator Diversity in Relation to Flowering Plant Phenology in the Western Mindanao State University Experimental Forest Area, Upper La Paz, Zamboanga City, Philippines

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Insect pollinators are crucial for maintaining forest biodiversity and ecosystem stability. This study aimed to examine the temporal patterns of insect pollinator diversity in relation to the phenology of flowering plants within the Western Mindanao State University Experimental Forest Area (WMSU-EFA), Upper La Paz, Zamboanga City, Philippines. Field surveys were conducted monthly over a year, using sweep netting and pan trapping methods to sample pollinators, and phenological data on flowering plants were recorded, noting their timing and duration. A total of 85 pollinator species from 5 major insect orders were identified, with Hymenoptera being the most diverse. The Shannon–Wiener Index ( $H'$ ) indicated that pollinator diversity peaked during the main flowering season from March to June ( $H' = 3.87$ ) and was lowest during the non-flowering season ( $H' = 2.45$ ). A strong positive correlation ( $r = 0.78$ ) was found between flowering plant abundance and pollinator diversity, highlighting the significant influence of plant phenology on pollinator activity patterns. These findings emphasize the critical importance of synchrony between flowering plants and pollinators for ecosystem stability. Understanding these temporal dynamics is essential for developing effective conservation strategies to protect pollinator populations and ensure the long-term stability of forest ecosystems amidst changing climatic conditions. This study provides valuable insights that can inform conservation efforts, enhance biodiversity, and help mitigate the impacts of environmental changes on pollinator diversity and ecosystem health.

### 6.4. Estimation of Ploidy Level and Associated Traits Signifying the Positive Impacts of Genome Size Increase on Leaf Anatomical Traits of *Morus* spp.

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One of the fundamental aspects of evolutionary biology is understanding genome organization and its functional aspects that directly or indirectly act in concert with the adaptability of any organism. The evolution of chromosome size, number, and structure, as well as changes in DNA composition, suggest the high plasticity of nuclear genomes at the chromosomal level. Hence, chromosomal information on chromosomal complements bears significant importance in plant breeding and genetic studies, including genome analysis. Regardless of the prime focus on the mulberry crop, the understanding of its ploidy variation and ploidy-associated traits is lacking, which hinders the development of strategies for its systematic improvement and conservation. In the present research, with the execution of classical cytology, the chromosome numbers of 20 mulberry accessions (*Morus* spp.) have been estimated using shoot tip meristems. Chromosomal complements accounted for definitive intraspecific variation at ploidy levels such as diploid ( $2n = 2x = 28$ ), triploid ( $2n = 3x = 42$ ), tetraploid ( $2n = 4x = 56$ ), and hexaploid ( $2n = 6x = 84$ ) among the studied species (*M. alba*, *M. australis*, *M. bombycis*, *M. cathayana*, *M. indica*, *M. laevigata*, *M. latifolia*, *M. macroura*, *M. rotundiloba*, and *M. serrata*). Additionally, flow cytometry analysis was performed for the validation of chromosomal data. To identify ploidy-associated traits, different functional traits were studied. Principal component analysis (PCA) and heat map analysis indicate the positive impact of genome duplication on leaf anatomical

traits. Further consideration of below-ground traits will require in-depth understanding. Moreover, the present outcomes of intraspecific ploidy variation and identified ploidy-associated traits provide a platform and open windows for frontier omics approaches to improve the mulberry crop in the near future.

#### 6.5. Genetic Characterization of Primitive and Wild Wheat Species Using SSR Markers

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Wheat is one of the most important cereal crops, occupying a significant place in human nutrition. With the continuously increasing population, there is a need to increase food production by 70%. However, climate change is expected to decrease wheat production by 2050. One of the main reasons behind this is the decreased tolerance of modern wheat genotypes towards stressed environmental conditions. This can be attributed to decreased genetic diversity in modern wheat cultivars due to continued domestication and breeding process. It is thus necessary to increase the genetic variation of existing wheat genotypes. Wild and primitive wheat species are important genetic resources with a tolerance to different stress-related traits. However, in order to utilize them effectively in pre-breeding and breeding programs, it is necessary to know the variation among these genetic resources and to determine their genetic distance as compared to modern cultivars. Consequently, in this study, we evaluated the genetic polymorphism, genetic relatedness, and allelic differences of primitive, wild, and modern wheat genotypes employing simple sequence repeat (SSR) markers. The obtained results revealed high genetic polymorphism within and between the studied species. The employed SSR markers were efficient in identifying the genetic variation of different wheat species. Both the scatterplot and dendrogram clustered the genotypes into two main groups, with one containing the hexaploid genotypes and the other containing the tetraploid genotypes. The results and the genetic variation information obtained in this study can be used in ongoing pre-breeding and breeding programs and will be a guide for new breeding studies aiming to develop stress-resistant wheat genotypes.

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#### 6.6. *Aegilops* Species as a Source of Boron Toxicity Tolerance

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*Aegilops* species are distributed all around the world and successfully grow in regions suffering from severe heat, drought, diseases, and nutrient deficiency and toxicity. With their good adaptation to challenging environments and due to their being one of the closest relatives of modern wheat, they can be efficiently utilized for wheat improvement towards biotic and abiotic stress conditions. Several studies have reported the presence of beneficial traits, such as higher nutrient content, resistance to barley yellow dwarf virus, cereal aphid, Hessian fly, powdery mildew, and rust, as well as tolerance to abiotic stresses including salinity, drought, heat, etc., in different *Aegilops* sp. to facilitate modern wheat breeding. However, limited reports are available on the boron toxicity tolerance of *Aegilops* species. Boron toxicity stress restricts wheat agricultural production in different parts of the world,



especially in arid and semi-arid regions, including Turkiye. The stress largely affects the physiological, biochemical, and molecular mechanisms in plants, consequently reducing their growth and yield. Here, we report the diversity of different *Aegilops* species grown under high-boron growth conditions, focusing on their physiological and biochemical responses. It is extremely important to discuss and decipher how and why *Aegilops* can be a potential source of boron toxicity tolerance so that these species can be effectively used in future breeding programs targeting this agricultural problem.

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#### 6.7. Transcriptomic Changes in Boron-Tolerant *Triticum zhukovskiyi* Genotype Under High Boron Conditions

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*Triticum zhukovskiyi* is a hexaploid cultivated wheat form that was first reported by Upadhyya and Swaminathan in 1963. The species with an AAAAGG genome developed from the crossing of domesticated *T. timopheevii* (AAGG) with cultivated einkorn *T. monococcum* (AA), established a separate lineage (AAGG). Despite its restricted distribution, the *Timopheevi* lineage has previously been explored for resistance towards various biotic stresses such as stem rust, fusarium head blight, leaf rust, etc. However, there are limited reports on the abiotic stress tolerance of *T. zhukovskiyi*. Boron toxicity hinders wheat production around the world via yellowing and necrosis of leaves, limiting the plant's growth and development. In order to reduce this loss, it is necessary to identify diverse genetic wheat resources with greater tolerance towards high boron and understand the underlying mechanism that is responsible for making them boron-toxicity-tolerant. Here, we report a boron-toxicity-tolerant *T. zhukovskiyi* genotype and discuss how the physiological and biochemical response towards high boron is associated with molecular changes in plants under boron toxicity. Differentially expressed genes in boron-treated plants were found to be directly related to different biochemical pathways. However, it is important to confirm the functions of these genes, so that these can be further utilized in breeding programs to diversify the boron toxicity tolerance trait in modern wheat cultivars. The future prospects of efficiently utilizing this genotype and the obtained information will also be thoroughly discussed.

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#### 6.8. Unveiling the Rich Diversity of Epiphytic Bryophytes of Moist Temperate Deciduous Forest in the Western Himalayas of India

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Epiphytic bryophytes, which inhabit the branches and trunks of trees, play a salient role in the forest ecosystem. Their substantial biomass and ability to maintain canopy moisture contribute significantly to ecological functions. They serve as indicators of forest succession dynamics and help to compensate for nitrogen loss in forest environments. By colonizing tree bark, they retain and intercept water, providing favourable conditions for subsequent epiphytic vascular plants. The present study aimed to explore the floristic diversity of epiphytic bryophytes of the Western Himalayas, India. A thorough survey was carried out during the winter season (November 2023) across various sites within the study area, encompassing a range of elevation gradients. Different host tree species were selected

through stratified random sampling with strata classified into tree base, lower and upper trunk, and lower and upper crown. Epiphytic bryophyte samples were collected by employing  $10 \times 10 \text{ cm}^2$  quadrats. The samples were stored in zip lock bags and brought to the laboratory for further identification. The study revealed a remarkable diversity of epiphytic bryophytes in the Western Himalayan region. A total of 99 epiphytic bryophyte species distributed in 67 genera and 35 families were recorded. Among the 35 families, 28 families belong to mosses and seven families belong to the liverworts. The dominant families were Meteoriaceae and Mniaceae. On the contrary, only one species was recorded from Bruchiaceae, Leucodontaceae, Leucomiaceae, Orthostichellaceae, Pterobryaceae, Ptychomniaceae, Regmatodontaceae, Rhytidiaceae, Scapaniaceae, Sematophyllaceae, Cryphaeaceae, and Lesakeaceae. Moreover, *Quercus* and *Rhododendron* tree species were found to have the most diverse epiphytic bryophytes. This research observed a diverse array of epiphytic bryophytes, reflecting rich ecological complexity and providing various ecosystem services. Additionally, the study contributes to the Western Himalayan bryophyte flora and provides baseline data for future ecological and biodiversity conservation.

#### 6.9. Fern Diversity and Soil Characteristics in the Moist Temperate Deciduous Forest of the Indian Central Himalayas

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Ferns form a vital component of the vegetation of the Indian Central Himalayas (ICH). Comprehending winter-specific fern diversity is essential for understanding their adaptive strategies and informing conservation initiatives. This study aims to evaluate fern diversity in relation to soil attributes within the moist temperate deciduous forest of the ICH during the winter season, providing insights into the ecological dynamics and conservation status of ferns.

A comprehensive survey was conducted across various habitats in the ICHs during November 2023. Stratified random sampling ensured coverage of multiple microhabitats, including forest undergrowth, riverbanks, and shaded rocky areas. Fern specimens were collected and identified using morphological traits, with herbarium consultations for verification. Soil samples were taken from fern locations, and parameters such as soil pH, moisture, electrical conductivity, and elemental composition were analysed to assess their correlation with fern distribution. Multivariate statistical techniques were used to identify patterns in fern diversity and ecological preferences.

The survey recorded 23 fern species from 16 genera and six families, with Polyodiaceae and Pteridaceae being the most dominant despite seasonal constraints. Fern diversity peaked in shaded and moist microhabitats with slightly acidic soil (pH 5.08–6.20). Statistical analyses revealed a strong correlation between soil moisture, nutrient availability, and fern diversity, underscoring the importance of hydrological factors in winter fern distribution.

This study highlights the resilience and adaptability of ferns in the ICH during the winter season. The findings stress the importance of specific microhabitats and soil characteristics in supporting fern diversity under seasonal climatic constraints. Conservation strategies should focus on these key habitats to preserve fern diversity. Further research is recommended to investigate the phenological and physiological adaptations of ferns across different seasons, contributing to a comprehensive understanding of their ecological dynamics.

#### 6.10. Comparative Study of Rosmarinic Acid Content in Methanolic Extracts of Bulgarian *Thymus* Species, Section *Hyphodromi*

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Genus *Thymus* consists of around 250 species known for their therapeutic properties: antioxidant, anti-inflammatory, expectorant, and carminative. Twenty-one species occur

naturally in Bulgaria, with eight belonging to the section *Hyphodromi*: Bulgarian endemics: *T. jalsianus* Stoyanov & Marinov. and *T. perinicus* (Velen.) J alas; Balkan endemics: *T. aznavourii* Velen. *T. comptus* Friv.; *T. atticus* Čelak (sub-endemic), as well as *T. leucotrichus* Halácsy *T. striatus* Vahl. and *T. zygioides* Griseb., all widespread. Due to the species' limited distribution and the overall complexity of the genus, extensive studies are lacking. Rosmarinic acid is a phenolic compound with highly valued biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Many thyme species are reported to be sources of this polyphenol, showcasing their potential beneficial properties. This study aimed to estimate the rosmarinic acid content by a quick, accurate, and readily available method such as HPTLC in the methanolic extract of the *Thymus* species section *Hyphodromi* distributed in Bulgaria. Plant materials were collected from the natural localities of the studied taxa. The quantification of rosmarinic content was achieved using HPTLC silica gel 60 F<sub>254</sub> plates. The mobile phase was chloroform/ethyl acetate/formic acid (50:40:10). The compounds were visualized by spraying with 'Naturstoffreagenz A' reagent. The fluorescence emission of rosmarinic acid was recorded under UV radiation at 336 nm, using a digital camera. The images were analyzed by QuantiScan 2.1 Biosoft software. The results shows that the content varied between 1.20 and 2.50 µg/g dry weight. *Thymus atticus* and *T. jalsianus* extracts were found to be the richest sources of rosmarinic acid, while those of *T. comptus*, *T. aznavouiri*, and *T. striatus* showed moderate levels. The obtained data showed *Thymus atticus* and *T. jalsianus* as promising for cultivation when plant material with a high rosmarinic acid content is needed.

#### 6.11. Taxonomic Revision of Genus *Annona* Native to Africa Using Selected Barcode Loci (*matK*, *trnL* and *rbcL*)

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The genus *Annona* is a genus of flowering plants in the sugar apple family, Annonaceae. It is the second largest genus in the family after *Guatteria*, containing approximately 166 species of mostly Neotropical and Afrotropical trees and shrubs. It is a traditional food plant in Africa. There have been some taxonomic difficulties in identifying the species within this genus using morphological traits alone. To understand the taxonomic affinity of this genus, there is a need for molecular discrimination. This study was therefore designed to check the possibilities of molecular markers in identifying the species within the genus *Annona*.

Thirty-three sequences (33), which represents three barcode loci (*matK*, *rbcL* and *trnL*), were retrieved from the NCBI website. They were combined and aligned using the ClustalW component in MEGA 11 software. The aligned sequences were evaluated, and this provided the genetic characteristics, match identification rate, barcoding gaps, haplotype diversity and nucleotide diversities of the markers. A phylogenetic tree was also constructed using the Neighbor-Joining tree-based method of MEGA 11.

The results showed the number of variable sites for all single and combined markers ranging from 2 (*trnL*) to 1077 (*matK* + *trnL* + *rbcL*) and the Parsimony informative sites ranging from 2 (*rbcL*) to 630 (*matK* + *trnL* + *rbcL*). The average intraspecific distance was discovered to be zero (0) for all single and combined markers, while the interspecific distance ranged from 0.002 (*rbcL*) to 0.990 (*matK* + *trnL* + *rbcL*). The results of this study showed that *matK* + *trnL* had the highest for best match (71.42%), while *matK* had the highest value for the best close match (38.46%). The phylogenetic tree generated revealed that only three species (*Annona glabra*, *A. senegalensis* and *A. stenophylla*) are paraphyletic.

### 6.12. Microsatellite Analysis Reveals Genetic Variation in 'Yerba Mate' Trees from Argentina

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*Ilex paraguariensis* A. St. Hil. is a perennial, dioecious tree native to southern South America. It has great socio-economic importance in the region due to its use for the preparation of 'mate' beverages. In this study, individuals from a remnant of subtropical forest held in the Campo Anexo Manuel Belgrano (CAMB, San Antonio, Misiones, Argentina) were genetically characterised. Leaves from 113 adult plants from 11 subpopulations were collected, covering a total area of 15.5 km<sup>2</sup>, aiming to represent the potential variability within it. Subpopulations are, on average, 1.5 km apart from each other, and plants within each subpopulation are separated by at least 5 m. Total genomic DNA was extracted using commercial kits, and six nuclear microsatellite loci were PCR-amplified under optimised conditions. Allele sizing was accomplished by automated fragment analysis and comparison with molecular weight standards. The frequency data matrix was analysed in GenAlEx. Genetic distances were obtained using Nei's index with the poppr library within the R environment; a Neighbour-Joining (NJ) network was generated using the ape library. The distribution of allelic variation showed 53 alleles (average: 8.83 alleles per locus). The average heterozygosity values were  $H_o = 0.541$  and  $H_e = 0.604$ , Shannon's information index resulted in 1.303, on average, and the Fixation Index was 0.097. These values are comparable to those reported for natural populations from southeastern Brazil and 1.96 times higher than those recorded for Uruguayan populations, which represent the southernmost species distribution tail. There was no clustering of individuals according to the sampled subpopulation in the NJ network. The study results reveal intermediate genetic variability in CAMB wild 'yerba mate' plants, but this was similar to that reported for the 'yerba mate' centre of diversification.

### 6.13. Plant Diversity and Agroecosystem Service Provision in Olive Orchards in Crete, Greece: The Effect of Agricultural Management Systems and Agroecological Zones

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The agricultural expansion and intensification of farming practices have deteriorated natural and agricultural ecosystems worldwide. Olive orchards cover the 1/4 of the agricultural land in Crete and about the 20% of the Cretan landscape, contributing largely to the island's topography, economy and cultural heritage. The management of spontaneous vegetation with sustainable/agroecological practices can be an important measure to mitigate the adverse effects of intensive farming in olive orchards, providing several agroecosystem services. Vegetative soil cover and plant diversity are directly related to biodiversity conservation, soil fertility, water quality and quantity, pest management, landscape conservation, outdoor recreation, climate regulation and resilience to climate change. Plant diversity and the effect of management systems and agroecological zones were studied in six paired conventional and organic olive orchards in Crete. The orchards are located at two agroecological zones (hilly and plain). In each orchard, the floristic diversity was determined in 3/ha monitoring stations (64 m<sup>2</sup> area) for a 1-year period. The effects of management system and agroecological zone on floristic diversity were determined with

biodiversity indices and univariate comparative analyses. In total, 270 species of plants were identified, including 50 edible species and 40 legumes, indicating the importance of spontaneous vegetation for the provision of agroecosystem services. The agroecological zone shapes the floristic diversity of olive orchards, especially in the wet period, with a higher diversity expected to be present in the hilly zone. Plant diversity did not present significant differences between organic and conventional orchards. The variety of farming practices and the intensity applied among the orchards are responsible for the above result. Further analysis of the effect of each farming practice on floristic diversity is needed to determine which practices are the most suitable for the improvement of olive orchards' environmental performance.

#### 6.14. Current Knowledge of the Hydrophytes of Lake Texcoco, a Brackish Wetland in Central Mexico

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The presence of brackish and alkaline environments allows for the settlement of highly adapted flora in and around Lake Texcoco, a relic of the lake ecosystems in central Mexico. The lake's biodiversity, in particular its flora, shows a particular composition. In recent years, the conservation of this Ramsar wetland of international importance has increased, but the knowledge of the aquatic plants and their communities that inhabit this wetland is limited. For this reason, in this research study, we compiled the available literature published from 1957 to 2021 that includes major mentions of hydrophytes records in the lake. A floristic review was generated, with a richness of 97 species, of which 40 are strictly aquatic, 23 semi-aquatic and 34 tolerant. Of these species, 23 are considered halophytes, 46 are freshwater plants and 28 are tolerant to salinity. Some of these species exist only in brackish wetlands; thus, in Mexico's central basin, they occur only in Lake Texcoco. Also, we found seventy-three native species—eight endemic and one under a national protection category—and sixteen alien species, of which six are invasive. This floristic compilation is proposed as a reference for future works on the diversity and conservation of aquatic plants in this lake remnant to increase the knowledge of a rare and threatened ecosystem in central Mexico.

#### 6.15. Genome Size Variation in Peruvian Andean Maize Landraces

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Maize (*Zea mays* ssp. *mays*) showed intraspecific variability in DNA content. This variation ranges from 40% to 58%, and negative correlations between genome size and cultivation elevation have been detected in Andean landraces from Northwest Argentina and Bolivia. These variations have been attributed to variability in the percentage of heterochromatin and the presence of B chromosomes. In order to investigate if variation in DNA content is also observed in landraces from the secondary center of the diversification of maize, genome size was estimated in nine Andean landraces from Cuzco and Abancay, Peru, which are cultivated between 2450 m.a.s.l. and 3483 m.a.s.l. Inter- and intraracial

variations in DNA content were observed, although with a lower percentage of variation (13.21%) than that reported by other authors. The mean 2C value was  $5.14 \pm 0.42$  pg, with a range from  $4.54 \pm 0.05$  pg estimated in the landrace Kculli to  $5.44 \pm 0.14$  pg found in the landrace Confite Morocho. No correlation between genome size and cultivation elevation was found. The fact that we observed much smaller genome size variation values than those previously reported, as well as the lack of correlation with cultivation elevation, could be due to the narrow elevational cline sampled. Future studies will be conducted expanding the elevational range. The variation in genome size found here will be discussed in reference to the variability in heterochromatin content and B chromosomes that these Peruvian landraces possess.

#### 6.16. Biodiversity of Endophytic Fungi in the Genus *Miliusa*

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The genus *Miliusa* consists of about 60 species. So far, India has been home to twenty-three species and one variant of the genus. With 71 percent of the species being endemic, the genus is phylogeographically significant in the Western Ghats, making it more important from a conservation standpoint. These plants are utilized for their antibacterial, anticancer, anthelmintic, and antiparasitic properties, and also as pesticides. However, only a few species have been thoroughly investigated. The genus *Miliusa* includes a variety of secondary metabolites, according to a large body of evidence. Alkaloids, geranylated homogentisic acid, flavonoids, lignans, neolignans, terpenoids, acetogenins, styryls, lactones, phenolics, amides, alcohols, and furfural derivatives are some of the compounds found in these plants.

Endophytic fungi represent an important and quantifiable component of fungal biodiversity and affect plant community diversity.

The main objective of the current research was to study the biodiversity of endophytic fungi from the stem, leaves, and bark of *Miliusa velutina* var *deviayarina*.

Small parts of stems, leaves, and bark were crushed in order to expose endophytes and subculture them on potato dextrose agar and Sabouraud agar for one week at room temperature and further subculture them many times in order to obtain a pure culture.

Some dominant endophytic fungi like *Aspergillus niger* Tiegh., *Colletotrichum siamense* Prihast., and *Diaporthe* sp. were discovered by morphology and molecular identification.

Endophytic organisms isolated from this plant were found to secrete many useful enzymes, such as lipase, amylase, pectinase, asparaginase, tannase, etc. Fungal colonies were grown on various media to check their enzymatic activity. This endophytic fungal biodiversity has the ability to produce natural and potentially bioactive compounds for different processes. These endophytic fungi have shown promising antimicrobial activity against certain groups of microorganisms, including Gram-positive and Gram-negative bacteria.

This research work highlights the significance of endophytic fungal biodiversity derived from medicinal plants as a source of innovative and bioactive chemicals.

#### 6.17. Evaluation of Moroccan Autochthonous Grapevine Varieties Diversity (*Vitis vinifera* L.) by Linear Morphometric Traits of Seeds and Ampelometric Traits of Leaves

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The cultivation of (*Vitis vinifera* L.) is well rooted in the traditions of the Maghrebian peasant populations in general and Moroccan in particular, it is the result of the ancient

tradition of the cultivation of the vine in the country, which began in the Roman period. Grapevine varieties are characterized by significant variability in seed and leaf morphology, and they are generally used to describe *V. vinifera* varieties.

The present study aims to evaluate the diversity of 27 autochthonous Moroccan grapevines, via the ampelometric and the linear morphometric traits. We sampled 270 grapevine seeds and 270 leaves belonging to the Moroccan varieties. The study is undertaken using seven characters of linear morphometry. According to the leaves, nine ampelometric traits were established following the OIV.

An ANOVA test is performed to highlight the most discriminating parameters. However, a principal component analysis (PCA) has allowed the morphometric parameters of the seeds to group the majority of the Middle and High Atlas varieties, while the parameters relating to the leaves have allowed the varieties from the Western Rif to be grouped. The results obtained from the different populations of seeds and leaves of autochthonous varieties of Morocco confirmed that the set of morphometric measurements used have an undeniable discriminating power and showed the presence of an important variability not only inter-varietal but also intra-varietal of the studied varieties. However, it has been inferred that seed and leaf parameters are weakly correlated, so it's important to carry out both complementary studies.

#### 6.18. Life Cycle Assessment of Potato Production in Northern Morocco

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In Morocco, potato production is set to rise from 1641 thousand tonnes in 2021 to 1768 in 2022, an indication of increasing consumer demand. The Tanger-Tétouan Al-Hoceïma region accounts for 15% of national potato production, with almost 82% of production in the province of Larache. Potato cultivation can have a range of impacts on the environment due to its high fertilizer requirements compared with other crops, particularly with regard to percentages of nitrogen (N), phosphorus (P) and potassium (K). Otherwise, agricultural practices commonly used to grow potatoes in the study area include the following: field preparation, the incorporation of organic fertilizers into the soil, seeding, fertilization, irrigation, pesticide spraying and plant protection. In order to determine the environmental impacts associated with potato cultivation in the Larache region, we used the Life Cycle Assessment (LCA) methodology using openLCA software 2.2, focusing on the following impact categories: Acidification (AP), Global Warming (GWP), Eutrophication (EP), Human Toxicity (HT), Freshwater aquatic ecotoxicity (FAE), Terrestrial ecotoxicity (TE), cited in the CML method, and USEtox to identify the following categories: freshwater ecotoxicity, human health—carcinogenic and human health—non-carcinogenic.

A “cradle-to-gate” approach was adopted, taking into account the type of technological practices followed, the machinery used, its operating time, and the quantity of potato plants, fertilizers, pesticides, fuels used and water for a functional unit of 1 hectare of cultivated potato. The results show that the main contributors to the environmental impact of potato cultivation are the relatively high consumption of diesel and machinery.

### 6.19. Reprogramming of Proteins and Modulation of Antioxidant Enzymes in Alfalfa (*Medicago sativa*) Seedlings

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During the process of germination, seeds use up their protein reserves and experience various physiological and biochemical changes, leading to stress. This study examined the impact of Abscisic acid (ABA) and nanoparticle treatments on the regeneration of proteins consumed during the germination of alfalfa seedlings, as well as the activities of antioxidant enzymes in germinating seeds. Specific antioxidant enzymes play crucial roles in plant growth and development at different stages of growth and conditions. Alfalfa seeds were germinated and treated with Titanium dioxide (TiO<sub>2</sub>) and activated charcoal after 48 h of germination. The protein was extracted from seedlings that were 5, 7, and 10 days old. The analysis showed that the seedlings treated with ABA and TiO<sub>2</sub> had a higher protein content than other treatments but lower than dry seed, which serves as a control. An SDS-PAGE examination revealed the reappearance of some protein bands in seedlings treated with ABA and TiO<sub>2</sub> after 5, 7, and 10 days of germination. The activities of catalase and peroxidase showed an increase as the seedlings grew older and were shown to be higher in seedlings treated with ABA and TiO<sub>2</sub>. The findings of our study indicate that the application of ABA and TiO<sub>2</sub> alters the expression of proteins and increases the activity of antioxidant enzymes in young alfalfa seedlings, perhaps leading to an enhanced ability to withstand stress. These discoveries have implications for the growth of young plants and the enhancement of agricultural yields.

### 6.20. The Floristic Composition of Some Botanical Gardens in the Fayoum Depression, Egypt

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The Fayoum Depression (FD) is considered one of the world's oldest agricultural cities and the most fertile agrarian terrain in Egypt. The topography of FD is comparable to that of Egypt, with Qarun Lake situated on Fayoum's northern coastline, resembling Egypt's northern border with the Mediterranean Sea, and the Bahr Yusuf Canal, referred to as FD's backbone, being akin to Egypt's Nile River. This article discusses the cultivated, tree, and weed flora composition of the plants growing in four common gardens in the FD (Fayoum University Gardens—FUGs, Fayoum Plantation International Garden—FPIG, Fayoum Governorate Club—FGC, and Fayoum Zoo—FZ). Two hundred sixteen species and one hundred fifty-one genera representing 58 plant families have been identified. Asteraceae, Moraceae, and Fabaceae are the richest families of the region's floristic composition. Unexpectedly, perennial species (81%) were dominant over annual species (19%). This is a prominent feature in the FD, where perennial species may be more tolerant of climatic changeability than annual species. Six life form categories were found; Phanerophytes (46.5%) were the most common life form, followed by Hemicryptophytes (38%). Furthermore, there were four primary phytogeographical plant groups: worldwide, bi-regional, pluri-regional, and mono-regional. The largest participation rates were seen in the bi-regional and mono-regional categories, with 21% and 53%, respectively. Twenty-seven species (13%) were found to be native to the Saharo-Arabian region. The current results of the flora survey include information on the life form, chorology, and lifespan of the area's wild and cultivated plants, as well as information on their collection, identification, and documentation.



6.21. *Unveiling Diversity: Morphological and Biochemical Insights into Datura metel L. Accessions*  
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*Datura metel* L., a medicinal plant in the Solanaceae family, is known for its narcotic properties and a range of physiological effects. This study investigates the diversity of 25 *D. metel* L. accessions using twelve morphological and seven biochemical parameters. The aim is to enhance our understanding of the plant's diversity and its potential application. Twenty-five accessions of *D. metel* L. were cultivated under standardized conditions. Morphological traits, including plant height, leaf length, and flower dimensions, were measured. Biochemical analysis of key compounds such as scopolamine, hyoscyamine, and atropine in the seeds and leaves was performed using High-Performance Liquid Chromatography (HPLC). This study revealed significant variability in both morphological and biochemical traits among *D. metel* L. accessions. Apigenin (66.28%) and atropine (44.19%) exhibited the highest coefficients of variance, while flower length (8.60%) and scopolamine (7.38%) displayed the lowest. HPLC results demonstrated notable discrepancies in biochemical levels across accessions. Cluster analysis classified the accessions into four groups, showing partial alignment with their origins. This study provides valuable insights into the physiological effects and properties of *D. metel* L., highlighting its morphological and biochemical diversity. These findings could contribute to the plant's enhanced agricultural and medicinal applications, emphasizing the importance of precise biochemical quantification for therapeutic advancements.

6.22. *Diversity of Wild Edible Plants (WEPs) in the Bhaderwah Region of Jammu and Kashmir, India*

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Wild Edible Plants (WEPs) are those plants which are not cultivated but are collected from their natural habitat for human consumption. Studies on WEPs in different parts of the world have revealed that these plants can be utilized as good sources of minerals and other micronutrients which are commonly lacking in regular human diets. Many of these plants also have immense therapeutic properties and nutraceutical potential. The current study was carried out in the Bhaderwah region of Jammu and Kashmir, India, which is a predominantly rural area inhabited by some tribes, in addition to various ethnic groups. The region is mountainous and rich in floral diversity. The local people consume many wild plants for their health benefits. The diversity of these plants in the region was studied and some of these plants were evaluated for their nutritional value and for the presence of heavy metals. WEPs were collected from the study area by carrying out field trips. Ethnobotanical investigations were also conducted. The collected plants were identified. Herbarium sheets of the identified specimens were prepared and subsequently submitted to the University of Jammu Herbarium. For nutrient analysis, standard methods of the Association of Official Agricultural Chemists (AOAC) were used. For mineral analysis, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and XRF were used. This field study indicated a rich diversity of WEPs in the region. Sixty plants belonging to 31 families were identified. Some of the evaluated plants were found to have good nutrient and mineral content, with limited anti-nutrients.

It can be concluded that the study area has a rich diversity of WEPs, some of which are high-altitude plants with immense medicinal properties. WEP diversity in the region needs to be explored further and evaluated for important phytochemical and nutraceutical potential.

### 6.23. The Impact of Sunflower (*Helianthus annuus*) Diversity on Ecosystem Functioning

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This study explores the role of sunflower (*Helianthus annuus*) diversity in enhancing ecosystem functioning. By examining various sunflower cultivars, we assessed their impact on soil health, pollinator attraction, and overall biomass production. Our findings indicate that higher genetic diversity within sunflower populations leads to improved soil nutrient cycling and increased resistance to pests and diseases. Sunflower populations with higher genetic diversity exhibit increased resistance to pests and diseases, promoting healthier crops. By maintaining genetic diversity, sunflower farmers can foster more resilient and productive crops, reducing the need for external inputs. Ultimately, our findings suggest that preserving genetic diversity is crucial for sustainable sunflower production and ecosystem health. Additionally, sunflower plots with diverse genetic makeup attract a wider range of pollinators, including bees, butterflies, and other beneficial insects. This increased pollinator diversity leads to enhanced pollination efficiency, resulting in higher seed production and yields. By cultivating diverse sunflower populations, farmers can create a more welcoming environment for pollinators, promoting ecological balance and boosting crop productivity. These results underscore the importance of maintaining genetic diversity within crop species to promote sustainable agricultural practices and ecosystem resilience. This study highlights the potential benefits of incorporating diverse sunflower cultivars into farming systems to enhance both productivity and environmental health.

### 6.24. Multivariate Analysis of Peruvian Maize Races

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Maize represents one of the most important staple crops in the Peruvian highlands. The abundant morphological diversity found in Peruvian maize from the highlands was classified by a previous published work into six groups: (i) primitive races—PRs, (ii) anciently derived or primary races—ADPRs, (iii) lately derived or secondary races—LDSRs, (iv) introduced races—INTRs, (v) incipient races—INCRs, and (vi) imperfectly defined races—IDRs. To date, Peruvian maize races have not been further examined. Here, we conducted multivariate analyses using the publicly available dataset of 124 morphological quantitative characters (ear, plant, leaf, tassel, pericarp, cob, endosperm, and kernel) for 43 Peruvian maize races scored to classify them in an attempt to provide additional evidence for that grouping and for determining congruence with our molecular study. Principal component analysis demonstrated that the first two components (CP1 and CP2) summarized 23.03% of the total variance. The top three variables that contributed more to CP1 were the leaf width, tassel length of the branching space, and tassel number of the primary branches, while the ear diameter base, ear internal diameter, and ear cupule width contributed more to CP2. Principal component analysis grouped all primitive races. The other races did not show a consistent clustering. On the other hand, most of the LDSR, INT, INCR, and IDR groups formed a single group. Our hierarchical clustering analysis using

the average method grouped all PRs, except Kculli, in a single clade. In addition, most of the ADPRs were grouped in two clusters. These results are in partial agreement with our molecular work. Further research is needed to determine the phenotypic plasticity of the races of Peruvian maize, considering that these landraces are in constant evolution in the Andes. We expect our work will stimulate other researchers to conduct maize germplasm characterization. We aim to use this precious genetic resource to alleviate poverty in the Peruvian Andes.

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