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Conference Report

Abstracts of the 1st International Online Conference on Biology †

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Abstract

The 1st International Online Conference on Biology (IOCBI 2026), held from 10 to 12 February 2026, brought together researchers from around the world to share and discuss recent advances across a wide spectrum of biological disciplines. Organized under the auspices of the journal *Biology* (MDPI), the conference provided an open, interactive forum for scientific exchange in a fully online, accessible format. The scientific program encompassed key areas of contemporary biology, including evolutionary biology, ecology, conservation biology, infection biology, zoology, marine biology, and plant sciences. Through keynote lectures, invited talks, oral communications, and flash presentations, the conference highlighted both fundamental questions and emerging challenges that define current biological research. Beyond the diversity of topics, IOCBI 2026 underscored the increasing need for integrative approaches in biology. The contributions collected in this volume reflect a shared effort to connect processes across different levels of biological organization—from molecular systems to ecosystems—revealing life as a complex and evolving network of interactions. This perspective is essential not only for advancing fundamental knowledge but also for addressing pressing global challenges in health, biodiversity, and environmental change. All accepted abstracts included in this volume of *Biology and Life Sciences Forum* provide a citable record of the work presented at the conference and illustrate current trends and future directions in the biological sciences. We sincerely thank all authors, speakers, reviewers, and organizers for their valuable contributions, which made IOCBI 2026 a successful and stimulating scientific event.

Keywords: biological complexity; climate change; global biodiversity; host-pathogen interactions; plant adaptations

1. Evolutionary Biology

1.1. The Price of Path: Do Snake Roadkills in Colombia Reflect Phylogenetic Trends and Relate to Functional Traits?

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Roads are essential infrastructure for human development. However, they generate strong ecological impacts and represent a growing threat to wildlife populations by causing the death of millions of wild animals each year. Snake mortality from roadkill is of particular

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concern because snakes play fundamental ecological roles and yet remain poorly studied from both ecological and evolutionary perspectives, especially in Neotropical countries. In this study, we evaluated the association between snake roadkill records in Colombia and certain functional traits, while accounting for their phylogenetic relationships. To do so, we compiled records from databases, collections, and literature, calculated values of evolutionary distinctiveness (ED) at both global and national scales, estimated the potential loss of phylogenetic diversity (PD), and assessed the presence of phylogenetic signal in the incidence and occurrence of roadkill. We employed Phylogenetic Generalized Least Squares (PGLS) and Phylogenetic Generalized Linear Models (PGLM) to evaluate the relationship between functional traits (e.g., body length, coloration, microhabitat, activity pattern, and distribution range) at two levels: species recorded as roadkilled and the entire set of snake species present in the country. We found 1301 roadkill records for 94 species, mainly from the families Colubridae and Boidae, representing a PD = 2.007 Ma (41% of the national PD = 4.865 Ma). At the level of species recorded as roadkilled, no phylogenetic signal or association with the evaluated functional traits was detected, although the number of records increased with broader distribution ranges. On the other hand, considering all snake species present in the country, roadkill incidence was higher in those with conspicuous coloration, larger body size, and broader distribution ranges.

1.2. Assessing Protein Structural Changes Caused by Missense Variants via Molecular Dynamics Simulations

Daniela Barbosa [1,2], Fernanda Li [1], Carlos Ariel Yadró Garcia [1], Sana Bashir [3], Sarra Bin Younes [1], José Rufino [4,5], Raquel Martín-Hernandez [6], Carlos Seiti Hurtado Shiraishi [1], Rui Abreu [1], Ana R Lopes [1], M. Alice Pinto [1] and Dora Henriques [1]

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Understanding the impact of single-nucleotide polymorphisms (SNPs) on protein structure is a critical challenge in functional genomics. We developed a scalable computational pipeline to evaluate SNP effects on protein stability, flexibility, and dynamics, using genomic data from 1467 adult male honey bee drones (*Apis mellifera*) across 25 countries and 18 subspecies, sourced from the MEDIBEES project. High-stringency variant calling identified SNPs for analysis.

Wild-type structures were predicted using AlphaFold2, SWISS-MODEL, and AlphaFold Protein Structure Database models, ranked by confidence metrics (pLDDT, DOPE, TM-score). AlphaFold2 models showed high internal consistency (average TM-score 0.9975 $\pm$ 0.0015) and similarity to homology-based models (average TM-score 0.886 $\pm$ 0.076) and experimental structures (average TM-score 0.837 $\pm$ 0.001). SNP variants were generated via in silico mutagenesis using mutation-aware AlphaFold2, MODELLER (for homology modelling), and SWISS-MODEL. All structures underwent energy minimization to resolve clashes.

Molecular dynamics simulations (AMBER25, 100 ns each) under physiological conditions analyzed trajectories for solvent-accessible surface area (SASA), root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bonds (H-bonds), and dihedral angles (phi/φ, psi/ψ). These metrics revealed SNP-induced changes: in AlphaFold2 models, mean RMSD decreased from 1.81 Å (wild-type) to 1.58 ± 0.12 Å (mutants), suggesting improved stability; RMSF increased from 0.82 to 0.85 ± 0.05 Å, indicating higher flexibility; Rg decreased from 38.78 to 38.66 ± 0.09 Å, reflecting greater compactness; H-bonds reduced from 270.5 to 268.7 ± 3.6; and SASA declined from 54,139 to 52,946 ± 977 $Å^2$, implying reduced solvent exposure. In contrast, homology-based models (e.g., trRosetta) showed smaller perturbations (e.g., RMSD from 2.25 to 2.22 Å).

AlphaFold2 and AlphaFold Protein Structure Database wild-type models demonstrated the greatest sensitivity to SNP perturbations compared to homology models, with mutant structures maintaining high congruence to mutation-aware predictions (TM-scores > 0.99), underscoring the method's robustness for SNP effect modeling.

1.3. Chromosome Number Determination and Karyotype Evolution in Different Species of the Tribe Naupactini (Coleoptera, Curculionidae)

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The family Curculionidae shows a high incidence of parthenogenesis, particularly among broad-nosed weevils (subfamilies Entiminae and Cyclominae). Many parthenogenetic species are also polyploid, which may have important implications for their evolution and adaptation. The tribe Naupactini is a Neotropical group and one of the most diverse lineages within Curculionidae, including species of both ecological and agronomic relevance. However, cytogenetic studies of this tribe are scarce, and some reports of polyploidy remain uncertain or contradictory. Thus, the determination of the modal chromosome number (i.e., characteristic of this taxon) is highly relevant for understanding both the evolutionary history and genetic diversity of these species. The main objective of this study was to establish the chromosome number of different Naupactini species by analyzing spermatogonial meiosis, and subsequently to propose hypotheses on karyotype evolution. Between 5 and 10 meiotic preparations were examined for *Naupactus dissimulator*, *Naupactus xanthographus*, and *Pantomorus postfasciatus*, using 5% Giemsa staining. The modal chromosome number was determined: the first two species presented 2n = 22, while the latter showed 2n = 26. At least two possible evolutionary mechanisms were proposed to explain the observed karyotype variation: chromosome fission or fusion. Overall, these cytogenetic studies provide valuable insights into genetic diversity, the evolution of polyploidy, and the chromosomal evolutionary history of these agronomically important species, contributing to a better understanding of their biology and potential management strategies.

1.4. Decoding the CYP3 Landscape in Apis mellifera: Allelic Diversity, Detoxification, and Beyond

Fernanda Li [1], Daniela Lima [1,2], Sana Bashir [3], Carlos Yadro-Garcia [1], Dirk de Graaf [4], Lina De Smet [4], Gilles Verbinnen [4], Annelise Rosa-Fontana [1], José Rufino [5], Raquel Martín-Hernandez [6], Maria Alice Pinto [1] and Dora Henriques [1]

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The western honey bee (*Apis mellifera*) is an essential pollinator that is facing global colony losses, caused by multiple factors, including pesticide exposure. Like other insects, honey bees rely on detoxification pathways to metabolize xenobiotics into less toxic or readily excretable forms. These pathways are central to insecticide resistance and are shaped by genetic variation. In honey bees, Cytochrome P450 monooxygenases (CYP) genes of clade 3 encode proteins are central to xenobiotic metabolism, but their allelic diversity across natural populations has never been systematically characterized.

We analyzed whole-genome sequencing data from 1467 drones (haploid males) spanning 25 countries and 18 subspecies across all 4 major evolutionary lineages. Targeting all 28 CYP3 genes alongside 18 housekeeping controls, we identified 5247 single-nucleotide polymorphisms (SNPs) and calculated allele frequencies, haplotype diversity, and fixation index (F_{ST}). In addition, all variants were functionally annotated to evaluate their potential effects on protein function.

Our analysis revealed substantial heterogeneity in allelic diversity within the CYP3 clade. Genes implicated in xenobiotic detoxification—particularly CYP9Q3—displayed extensive allelic diversity, likely reflecting diversifying selection. Notably, we detected a previously reported CYP9Q3 haplotype conferring neonicotinoid sensitivity at low frequencies across Mediterranean populations, highlighting its potential utility for monitoring at-risk genotypes. Other genes mirrored the constrained mutational patterns of housekeeping genes, suggesting conserved physiological roles beyond detoxification. This functional specialization parallels observations in humans, where only a subset of CYPs bear the primary detoxification burden and are thus highly polymorphic.

Genetic diversity in *A. mellifera* is essential for colony resilience and adaptation. High CYP polymorphism likely enhances population-level tolerance to diverse environmental and dietary chemical exposures.

This work provides the first systematic assessment of CYP3 allelic diversity in *A. mellifera*, underscoring its fundamental importance for understanding pesticide responses and guiding protection efforts.

1.5. Evaluating the Effectiveness of Proxy-Based Environmental Variables in Detecting Genomic Responses to Pesticide Stress in Apis mellifera

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Assessing the impact of pesticide exposure on honey bees (*Apis mellifera*) is crucial for understanding the drivers of pollinator decline and the mechanisms underlying environmental adaptation. Honey bees play a key role in ecosystem functioning and agricultural productivity, but they are increasingly threatened by chemical stressors that can affect their health, behaviour, and genetic resilience.

Directly measuring exposure across large geographic scales is often logistically and financially challenging; therefore, environmental proxies—such as modelled pesticide use grids and land-use data—could facilitate this type of analysis by providing spatially explicit estimates of potential stressors. These proxies allow researchers to approximate exposure across landscapes, integrate multiple sources of environmental information, and combine them with genomic data to identify genetic variants associated with environmental pressures, even when direct measurements are limited.

To quantify pesticide exposure, we used two complementary approaches. The first measured direct pesticide exposure using INSIGNIA-EU's APIStrips (absorb pesticide in-hive strip) to quantify real-world pesticide residues. The second estimated the landscape-scale exposure integrating modelled pesticides-exposure grids from PEST-CHEMGRIDS to quantify the proportion of agricultural fields within 3 km radius of each colony using Corine Land Cover.

Leveraging these measured and proxy-based pesticide exposures, we analysed 102 whole-genome sequences across 33 European countries as part of the Better-B project. To identify candidate single-nucleotide polymorphisms (SNPs), three complementary Genomic–Environment Association (GEA) approaches were applied: SAMBADA (a spatial analysis tool), LFMM (latent factor mixed models), and Redundancy Analysis (RDA).

Even though the APIStrip measurements yielded a greater number of SNPs, both approaches converged on key detoxification and stress-response genes, including a transcript variant of sushi von Willebrand factor type gene. This overlap highlights the biological relevance of proxy-based signals, even with reduced statistical power, and supports their utility for large-scale genomic studies where direct sampling is infeasible.

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1.6. Experimental Reconstruction of Bacterial Fitness Landscapes

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Reconstructing interspecies relationships from microbial dynamics in natural environments and linking them to phylogenetic associations remains challenging in ecological and evolutionary studies. As an alternative approach, studying model bacteria under precisely controlled laboratory conditions can reveal ecological niche patterns and evolutionary consequences under defined environments. In this study, we evaluated bacterial growth to examine how environmental factors differentially affect proliferation processes and to construct experimentally derived fitness landscapes that may reflect natural adaptive processes. Multiple representative bacterial strains of broad phylogenetic diversity and distinct ecological backgrounds were used in the present study. These bacteria were cultured separately in hundreds of different media, which were composed of both purified chemical compounds and natural materials. This approach generated thousands of growth-rate and carrying-capacity values under a wide range of nutritional conditions. Despite strain-specific differences in growth, significant correlations were observed across media conditions. Moreover, clustering patterns based on growth profiles closely matched known phylogenetic proximity and biogeographical traits. It revealed that bacterial growth in response to laboratory-defined conditions was as conserved as evolution in nature, despite the significant differentiations between culture media and wild nature. Together, these findings demonstrated that laboratory-reconstructed bacterial fitness landscapes could serve as an experimental reference for bacterial ecological niches in natural environments. It indicated that functional traits such as growth dynamics can bridge trait–phylogeny relationships, providing a conceptual framework for experimental ecology and insights for microbial community design in the future.

1.7. Exploring a Deep-Learning Epigenetic Clock Based on an Interpretable Convolutional Neural Network to Unravel the Tick-Tack of Cellular Aging

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In the field of aging research, DNA methylation patterns have emerged as valuable epigenetic biomarkers for modeling the passage of time at the molecular level. Through so-called first-generation epigenetic clocks, it is possible to estimate an individual's chronological age with remarkable accuracy using the β-values of multiple CpG sites. Traditionally, these clocks have been built using machine learning models based on regularized linear regression (ElasticNet) for both feature selection and prediction. However, simple linear regression presents certain limitations, as such models are unable to capture nonlinear interactions between CpG sites or to model local dependencies among them. To overcome these constraints, recent approaches have explored deep learning methods capable of addressing these nonlinear and spatial relationships, although further research is still needed in this area. In this study, we compiled a large catalog of DNA methylation data from various tissues of healthy individuals differing in age, sex, and geographic origin. Using this dataset, we propose an approach based on a biologically interpretable convolutional neural network (CNN), which has been trained with images derived from methylation maps, in which CpG sites have been spatially organised according to their genomic position.

Our model aims to reduce systematic errors in chronological age estimation and to help identify new genomic regions involved in the epigenetic changes associated with aging.

1.8. Genetic and Evolutionary Mechanisms Shaping Reproductive Traits in Sheep: Implications for Breeding and Fertility

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Introduction: Sheep reproductive strategies serve as vital tools for studying evolutionary changes that occur within domestic livestock populations. The reproductive process of sheep depends on both natural genetic elements and environmental conditions which determine their evolutionary fitness. The research examines how sheep reproductive characteristics including fertility and sexual maturity age and litter numbers develop through natural selection and genetic diversity.

Methods: The research team performed genomic studies on multiple sheep breeds to discover reproductive success-related genetic indicators. Researchers gathered information about sheep mating patterns and their reproductive patterns throughout different seasons and their ability to survive until birth. The research used quantitative genetics to determine reproductive trait heritability and the existing evolutionary selection forces. The research examined how human intervention through selective breeding has transformed reproductive performance in domesticated sheep.

Results: The research discovered substantial genetic differences which affect reproductive characteristics between different sheep groups. The survival competition in certain environments selects for early sexual development and high reproductive capacity because these traits appear more strongly in such environments. Domesticated sheep breeds have shown better reproductive performance because of selective breeding programs. The study demonstrated that reproductive success differences between sheep populations result from both genetic drift and gene flow.

Conclusions: The research demonstrates that evolutionary processes directly influence the reproductive patterns of sheep. The genetic structure of reproductive characteristics demonstrates how natural selection together with human intervention through breeding programs influence sheep fertility levels. The research findings enable scientists to create better breeding strategies which enhance animal productivity and enable effective reproductive health management in both natural and controlled settings.

1.9. Investigating the Ancestral and Novel Functional Roles of Insl3: Gene Structure Parameters and Bioinformatics as Key Tools in Evolutionary Biology Research

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Introduction: This study investigates Insulin-like 3 (INSL3), also known as the Leydig cell-specific insulin-like peptide (LEY I-L), within the framework of evolutionary biology.

It examines key aspects of gene structure, such as the dynamics and intronic phase, and employs bioinformatics tools to explore both the ancestral and modern functional roles of Insl3.

Insulin-like 3 (INSL3) belongs to a superfamily of peptides, growth factors, and hormones that include insulin, IGF, and relaxin. INSL3 is primarily produced as a pre-prohormone in somatic cells of the gonads, particularly in the Leydig cells of the testes and theca cells of ovarian follicles. cDNA sequences of INSL3 have been isolated from several vertebrate species. The structure of the INSL3 gene is similar to that of insulin and relaxin, consisting of two exons and one intron.

Investigation Methods: We used in silico analyses to determine and compare the structure of the Insl3 coding gene across vertebrate, invertebrate and ancestral chordate genomes.

Results: Bioinformatics analyses presented here and performed on nearly all known insl3 genes and cDNA sequences from the database reveal that the insl3 gene structure is conserved among fish and various non-mammalian vertebrate species, although intron lengths differ.

The in silico results also provide evidence for the gain-of-function role of a single copy of the insl3 gene in mammalian testis development: zebrafish insl3 proximal promoters reveal the presence of the same point mutation in the binding site of a key transcription factor that affects the promoter activity of mouse and human INSL3. This mutation negatively impacts the transcription of the insulin-like 3 gene in Leydig cells. Since the gene for the binding factor is present in the zebrafish genome, the proposed hypothesis suggests that Insl3's new key role in testicular descent and in testis development might also arise from a point mutation in its DNA binding site on the promoter.

1.10. Investigating the Ancestral and Novel Functional Roles of Insl3: Gene Structure Parameters and Bioinformatics as Key Tools in Evolutionary Biology Research

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Results: Bioinformatics analyses presented here and performed on nearly all known insl3 genes and cDNA sequences from the database reveal that the insl3 gene structure is conserved among fish and various non-mammalian vertebrate species, although intron lengths differ. In silico resuls provide also evidence for a gain-of-function role of a single copy of the insl3 gene in mammalian testis development; zebrafish insl3 proximal promoter reveal the presence of the same point mutation in the binding site of a key transcription factor that affects the promoter activity of mouse and human INSL3. This mutation negatively impacts the transcription of the insulin-like 3 gene in Leydig cells. Since the gene for the binding factor is present in the zebrafish genome, the proposed hypothesis

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suggests that the key role of the new Insl3 in testicular descent and in testis development might also arise from a point mutation in its DNA binding site on the promoter.

1.11. Metref, an Auto-Updatable Web App for Reference/Representive Genomes and Their Complexity Metrics

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We have several open lines of investigation with informational complexity metrics in different sets of organisms within our group and, we needed a centralised solution to fit our needs. Of these complexity metrics, the most used in our analysis are BioBit and GS.

Genomic Signature (GS) (1) is an informational index anchored to k-mers. It is calculated by identifying the k that yields the most significant separation between the observed distributions of k-mer classes and their theoretical equifrequent expectations. This approach relies on the relative abundances of short oligonucleotides alongside the use of the chaos game representation applied to the genome. Meanwhile, BioBit (2) is a k-mer based metric of complexity that quantifies the deficit of entropy: the difference between the maximal possible entropy for a k-mer (e.g., in a random genome of equivalent length) and the genome's own calculated entropy for that same k-mer.

Because of that, a web app is proposed integrated with a database of reference and representative genomes as well as some genomic parameters and the calculation of the mentioned metrics.

A workflow was designed to treat and filter the genomes available on RefSeq. Genomes with assembly level "Chromosome" or greater and those with RefSeq category of "Representative genome" or "Reference genome" were kept. Then, two informational complexity metrics were calculated, as well as several genomic parameters. An auto-updatable system was designed so that every two months, a new version of the database is created with the newest genomes available. All this workflow was implemented using Python, Bash and Django.

1.12. Polygenic Adaptation of Reproductive Timing and Heat Resilience in Indigenous Sheep Across Aridity Gradients

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How complex life-history traits evolve under rapid climatic drying is a central question in evolutionary biology. Indigenous sheep maintained under pastoral systems experience strong, spatially structured selection on reproductive timing and thermotolerance, yet the genomic architecture and repeatability of these responses remain unclear. We sampled multiple native sheep populations spanning replicated aridity and heat-load gradients. Animals were phenotyped for puberty onset, litter size, lamb survival, and field thermophysiology (rectal temperature, respiration rate, body condition). Genomes were assayed

using high-density SNP genotyping complemented by RNA-seq from peri-pubertal granulosa cells. After modeling structure and relatedness, we combined genotype–environment association tests (LFMM, redundancy analysis) with selection scans (iHS, XP-EHH) and fitted reaction-norm animal models to quantify G × E. Expression QTL colocalization linked variants to gene regulation; GO and network enrichment interpreted pathways. Dozens of climate-associated loci clustered in modules tied to KNDy–GnRH signaling (e.g., KISS1/KISS1R, TAC3), ovarian steroidogenesis (STAR, CYP19A1), and cellular stress responses (heat-shock and vasoregulatory genes). Signals were dominated by soft sweeps and allele-frequency clines repeated across eco-clines, consistent with polygenic and convergent adaptation rather than single large-effect mutations. Reaction-norms revealed significant G × E for reproductive output, and polygenic indices derived from climate-associated SNPs predicted between-population variation in puberty timing and lamb survival under heat. Colocalization with granulosa-cell eQTLs implicated cis-regulatory shifts as a common mechanism. Reproductive timing and heat resilience in indigenous sheep evolve via repeatable, polygenic routes channelled through endocrine and stress-response networks. These results illuminate how selection structures complex traits in heterogeneous environments and provide evolution-informed targets for balanced improvement under warming, drying climates.

1.13. Rapid Evolutionary Adaptations to Parasitology: Insights from Basal Metazoans Belonging to the Myxozoa (Cnidaria)

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The complexity of biological phenomena can often be attributed to dynamic interactions among individuals within and across taxa. In parasitology, such interactions are commonly expressed as coevolutionary arms races between hosts and parasites, characterized by the necessity of continuous adaptation to maintain evolutionary fitness. Myxozoans represent a remarkable group of cnidarians that have undergone extreme morphological and genomic reduction, resulting in minute body sizes and highly compact genomes. These obligate parasites alternate between vertebrate and invertebrate aquatic hosts and are responsible for a variety of diseases affecting both cultured and wild fish populations. Despite their streamlined genomes, myxozoans retain a core set of conserved genes, while extensive gene loss and rapid, lineage-specific gene gains have driven their diversification and host specialization. These genomic innovations underpin several striking biological adaptations, including the complete loss of mitochondrial respiration in one lineage and the evolution of specialized surface proteins that facilitate blood-feeding and immune evasion in others. Here, we trace a decade-long journey of discovery that began with the sequencing of the first myxozoan genome in 2014. Subsequent research has profoundly expanded our understanding of the molecular and evolutionary mechanisms underlying parasitism in this lineage, shedding light on how myxozoans have achieved successful and stable genomic integration of new and transferred tools to adapt to their hosts—an extraordinary example of parasitic adaptation in one of the oldest groups of metazoan parasites on Earth.

1.14. Restoring Immune Visibility Through GcMAF Reactivation: A Multi-Layer Strategy Targeting Nagalase, Fibrin Architecture, and Tumour Field Coherence

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ECSBiohack | TeamMohamed, UK

Introduction: Tumours deploy advanced immune-evasion systems that suppress macrophage activation, disrupt antigen visibility, and create protective biochemical and

biofield structures. Among the most critical mechanisms is Nagalase-mediated inhibition of GcMAF, a key macrophage-activating factor. Additional layers of stealth, including fibrin encapsulation, suppressive exosomes, miRNA signalling, and bioelectric/quantum field synchronisation, further impair host recognition. This study presents an integrated therapeutic model that restores immune visibility by targeting biochemical, structural, metabolic, and field-level tumour cloaking mechanisms.

Methods: A multi-domain analytic framework was constructed by mapping tumour stealth pathways across enzymatic, fibrin-based, epigenetic, exosomal, miRNA, bioelectric, metabolic, and quantum-biological layers. Countermeasures were identified using literature-supported agents, biological activators, structured-water modulators, and field-disruption technologies. Key nodes of intervention included Nagalase suppression, GcMAF reconstitution, fibrin degradation, exosome interference, miRNA modulation, and coherence-breaking electromagnetic tools. A companion dataset (23 $\times$ 40 matrix) cross-mapped each stealth mechanism to therapeutic candidates, ECS interactions, GPCR/TRPV1 signalling, and biofield destabilisation points.

Results: Analysis identified Nagalase inhibition and GcMAF restoration as primary drivers of macrophage reactivation. Fibrin-targeting enzymes (nattokinase, serrapeptase) were predicted to dismantle tumour shielding, while mycological and botanical immunoregenerative agents enhanced antigen presentation. Exosome-disruptive compounds and polyphenol-based miRNA regulators reduced suppressive signalling. Electromagnetic and photonic tools demonstrated theoretical capacity for disrupting tumour coherence fields, improving immune visibility. Layered analysis revealed synergistic benefits when interventions were applied across biochemical and biofield domains simultaneously.

Conclusion: Restoration of GcMAF activity represents a foundational strategy for reversing tumour invisibility. Integrating enzymatic, metabolic, immunological, and quantum-biological interventions yields a unified therapeutic pathway capable of disrupting multi-layered cancer stealth systems. This framework supports development of personalised, multi-modal immunotherapeutics and informs future clinical translation.

1.15. The Role of Horizontal Gene Transfer in the Spread of Snowflake Forming Collagen Binding Aggregation Factors in Bacteria

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Snowflake Forming Collagen Binding Aggregation Factors (SFCBAF) are unique surface proteins originally described in lactic acid bacteria. These proteins determine the autoaggregation phenotype and are likely to be involved in biofilm formation and adhesion processes., which may serve as one of the mechanisms facilitating bacterial survival and dissemination in the environment. To date, only five collagen-binding aggregation factors of this type have been characterized in detail: AggL from *Lactococcus lactis*, AggE from *Enterococcus faecium*, AggLb from *Lacticaseibacillus paracasei*, AggLr from *Lactococcus raffinolactis*, and AggA from *Tetragenococcus halophilus*. In our research group, we have bioinformatically predicted and characterized new potential representatives of collagen-binding aggregation factors in phylogenetically diverse bacteria, including species beyond lactic acid bacteria, suggesting a possible role of horizontal gene transfer in their dissemination. In this study, we analyzed the genomic context of all genes encoding the predicted collagen-binding aggregation factors within a $\pm$7000 nucleotide window from the borders of the predicted gene sequence. This analysis revealed potential genetic elements that

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may participate in the mobility of these factors. In the majority of analyzed sequences, genes encoding transposases, integrases, or other proteins associated with mobile genetic elements were detected in the immediate vicinity of the aggregation factor genes. These findings emphasize the important role of horizontal gene transfer in the formation and dissemination of collagen-binding aggregation factors among bacteria of various genera and ecological niches, facilitating the spread of adaptive genes and enhancing bacterial plasticity in changing environments.

1.16. Trends in Genome Composition Across the Transition to Complex Multicellularity

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Genome architecture reorganized over evolutionary time to support complex multicellularity without a proportional increase in coding DNA. We conducted a cross-kingdom comparative analysis using high-quality RefSeq assemblies annotated by the NCBI Genome Annotation Pipeline, restricting the dataset to chromosome-level or complete genomes. We first computed key genomic variables for bacteria, archaea, and 694 eukaryotes (including 133 mammals, 77 birds, 169 fish, 187 arthropods, 128 plants, 130 fungi, and 53 unicellular eukaryotes), including genome size, gene content, and coding DNA content. Next, we fit scaling relationships among genomic variables and used PGLS to account for shared ancestry. Finally, we modeled the global trend using the mathematical form that best described these relationships. We identified clear regime shifts in composition. A transition near 40 Mb of gene content marked the shift from prokaryotes to multicellular lineages, beyond which coding DNA content scaled sublinearly with gene content and approached saturation. Prokaryotes exhibited near-proportional increases in the coding sequence with genome size, whereas eukaryotes showed progressive decoupling as the noncoding sequence expanded. Plants followed a distinct path, with gene content increasing sublinearly relative to genome size. Vertebrates, particularly mammals and birds, occupied compressed ranges of genome size and gene content consistent with strong compositional constraints; in these clades, coding DNA constituted as little as the 3% of total genic sequence. Together, these results revealed robust scaling laws and thresholds governing genome composition across the tree of life, quantifying how the expansion of the noncoding sequence dominated genomic evolution in the transition to complex multicellularity. On the one side, we computed summary statistics for the percentage of gene content relative to genome size, the percentage of coding relative to gene size, the percentage of coding relative to genome size, and the alternative splicing ratio across different taxonomic groups. On the other side, we also fitted a mathematical model that relates to the different variables.

2. Ecology

2.1. A Relict on the Brink: Modeling the Current and Future Distribution of Taxus baccata in Algeria Under Climate Change

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Taxus baccata L. (European yew) is a relict conifer with a fragmented distribution in North Africa, where its Algerian populations are restricted to humid and sub-humid mountain ranges. Increasing anthropogenic pressure and climate change threaten its persistence, making predictive modeling essential for conservation planning. We applied species distribution modeling (SDM) using the **sdm** package in R to estimate the current and future distribution of *T. baccata* in Algeria using an ensemble approach that combined Random Forest (RF), Support Vector Machines (SVM), and Boosted Regression Trees (BRTs). Occurrence data were compiled from GBIF and the literature, while current and future bioclimatic predictors were obtained from WorldClim. Future projections were generated under 2040 climate change scenarios. Model performance was consistently high (BRT: AUC = 0.99, TSS = 0.97; RF: AUC = 1, TSS = 0.99; SVM: AUC = 1, TSS = 0.99). Results revealed that *T. baccata* is confined to fragmented populations along Algeria's northern mountains and coast, with the eastern Tell Atlas as its main stronghold and only marginal presence in the west. The most influential predictors were maximum temperature of the warmest month (23.5%), mean temperature of the wettest quarter (11.6%), and precipitation of the warmest quarter (9.6%). By 2040, a significant contraction of suitable habitats is projected, with refugia persisting mainly in the north-east and at higher elevations of the Hauts Plateaux. These findings highlight the high vulnerability of *T. baccata* to climate change in Algeria. Protecting refugial habitats and promoting restoration efforts in fragmented populations will be critical to its long-term survival. Integrating predictive modeling into conservation policy can provide a robust framework for safeguarding this relict species under future climatic uncertainty.

2.2. Metagenomic Characterization of the Bacteriome in Simulium ochraceum from Former Onchocerciasis-Endemic Regions of Mexico

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Simulium ochraceum is recognized as the primary vector of human onchocerciasis in southern Mexico. It can also transmit other pathogens, including Mansonella ozzardii and vesicular stomatitis virus; however, its role in the transmission of bacteria remains unknown. Metagenomic approaches have enabled significant advances in the study of vector bacteriomes, with considerable impact on public health. These studies have identified symbiotic bacteria associated with reduced vector competence, as well as others with potential applications in biological control. However, the bacteriome of S. ochraceum remains uncharacterized.

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Therefore, this study examines the bacteriome of *S. ochraceum* from former onchocerciasis-endemic areas in Mexico, using a 16S rRNA metagenomics analysis. Adult female black flies were collected via humanlanding catches in November 2024 and morphologically identified using taxonomic keys. A pool of 50 individuals were used for genomic DNA extraction with the DNeasy Blood and Tissue Kit. Amplicons of the V4-V4 region were sequenced using the Illumina MiSeq platform. Bioinformatic analysis was conducted using EzBioCloud and CZ ID, which included the estimation and analysis of diversity, and estimation of bacteria associated with human and animal pathogens. The bacteriome was dominated by *Pseudomonadota*, with abundances of *Bacillota*, *Bateroidota*, and *Actinomycetota*. The identification of diverse bacterial taxa revealed the presence of potential human and animal pathogens, including members of the genera Escherichia, Streptococcus, Acinetobacter, and Rickettsia. Additionally, unclassified bacteria were detected, as well as microorganisms associated with environmental, plant-related, and insect-symbiotic niches. To our knowledge, this is the first report on the bacteriome of *S. ochraceum* on a global scale. The microbial diversity suggests a complex ecosystem with potential implications for public health, vector ecology, and environmental microbial dynamics.

2.3. Symptomatic and Biological Observations of Colomerus Vitis Under Changing Climatic Conditions

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Colomerus vitis (Pagenstecher) (Acari: Eriophyidae), commonly known as the erineum leaf mite, is an eriophyid mite that induces characteristic galls and deformities on the leaves and young shoots of Vitis vinifera cultivars. The present study aims to describe the symptomatic manifestations of *C. vitis* infestation, document its key biological features, and assess its potential ecological impact on vineyard ecosystems in eastern Romania. Observations were conducted during the active vegetative growth period across multiple cultivars and growth stages. Symptoms were systematically recorded through visual inspections and high-resolution photography, revealing leaf galls, curling, and shoot distortions of varying intensity depending on cultivar and plant age. Microscopic examinations confirmed the presence of mites within galls and allowed detailed observation of their morphology, spatial distribution, and population density, offering additional insights into their development and colonization patterns. Differences in symptom severity among cultivars indicated variability in host susceptibility and damage extent. Although *C. vitis* is not considered an invasive species in Romania, its growing prevalence and the potential for expansion under changing climatic conditions highlight its ecological significance. Recent data on the biology and damage patterns of *C. vitis* suggest that its increased prevalence, driven by warmer winters, may alter vineyard ecosystem dynamics and potentially increase the risk of transmitting viral pathogens, such as Grapevine Pinot gris virus (GPGV). These shifts emphasize the need for integrated pest monitoring and biodiversity-oriented management strategies. By documenting both macroscopic and microscopic features in a detailed and systematic manner, this study provides comprehensive descriptive information on the biology and symptomatic expression of *C. vitis*, contributing to a better understanding of arthropod–plant interactions and supporting future research on cultivar susceptibility, ecological resilience, and sustainable viticulture.

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2.4. Connectivity and Nursery Value for Mugil Curema (Perciformes, Mugilidae) in Four Estuarine Systems with Different Degrees of Anthropization in Northeast Brazil, Based on Elemental Chemical Signatures of Otoliths

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Estuarine ecosystems play an essential role as nurseries for several aquatic species. However, increasing anthropogenic influences compromise habitat quality, species recruitment and nursery value in these systems. From this perspective, the study examined estuarine-coastal connectivity of the *Mugil curema* species in four tropical estuaries with different levels of anthropogenic impact: Camaratuba, Mamanguape, and Paraíba and Gramame river estuaries, located in the northeastern Brazilian state of Paraíba. Based on the chemical analysis of the otolith cores, the elemental signatures of individuals from the same cohort, i.e., juveniles collected in 2022 (age 0+) and subadults (age 1+) collected in 2023, were retrospectively evaluated and compared, aiming to identify recruitment patterns and relative contribution of each estuary to coastal populations. One-Way Analysis of Variance (ANOVA), Multivariate Analysis of Variance (MANOVA) and Linear Discriminant Function Analysis (LDFA) revealed partial separation of multielement signatures spatially, with variations associated with local environmental conditions and the intensity of anthropogenic impacts. Elemental ratios such as Li:Ca and Ba:Ca stood out as important markers for spatial differentiation. The Paraíba River estuary stood out as the main nursery area for *Mugil curema*, presenting a high rate of self-recruitment through Multinomial Logistic Regression (MLR) and contributing to other estuarine and coastal areas. Despite being the most impacted estuary, especially due to the high concentration of lead (Pb:Ca), this system still plays an essential role in maintaining coastal stocks of the species, evidencing its ecological importance and the need for conservation policies. These results indicate that connectivity between estuaries and the coastal zone is shaped by local environmental and anthropogenic factors, and the nursery value of estuaries can vary considerably. The identification of key areas for recruitment and connectivity, such as the Paraíba estuary, provides important insights for fisheries management and conservation, especially in tropical regions under increasing anthropogenic pressure.

2.5. Contribution of the Functional Feeding Group (FFG) Approach to the Ecological Classification of Streams in Arid to Semi-Arid Regions: The Case of North African Wadis

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Introduction: North African wadis, though rare and often temporary, play a vital ecological and socio-economic role in arid to semi-arid regions. These fragile ecosystems face multiple natural and anthropogenic pressures, including prolonged droughts, irregular flows, water overexploitation, pollution, and habitat degradation. This study aims to analyze the structure and dynamics of the Functional Feeding Groups (FFGs) of benthic insects to assess the ecological quality of four wadis located in the Aurès region (eastern Algeria) and to determine whether the functional patterns observed are comparable to those reported in streams from temperate and tropical regions.

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Methods: Seasonal sampling was conducted from 2021 to 2022 across 48 stations.

Results: In total, 19,431 benthic insects belonging to 7 orders, 37 families, and 59 taxa, classified into 7 FFGs, were detected. In the unimpacted wadis, the benthic assemblages were characterized by the presence of several IBMWP-sensitive families, including Ephemeroptera (Baetidae, Caenidae), Trichoptera (Hydropsychidae, Sericostomatidae), and Coleoptera, which correspond functionally to shredders, filtering collectors, scrapers, and predators. These taxa are typically associated with good ecological status and stable habitat conditions in IBMWP-based assessments.

In contrast, impacted wadis were largely dominated by pollution-tolerant Diptera, particularly Chironomidae and Dixidae, which are classified as low-score taxa in the IBMWP system and correspond functionally to gathering collectors. The marked reduction or absence of sensitive IBMWP taxa in these sites is fully consistent with the functional diversity observed in temperate and tropical regions. FFG ratio analyses revealed clear differences between impacted and unimpacted wadis, highlighting variations in autotrophy/heterotrophy balance, habitat stability, and trophic structure.

Conclusion: Overall, the Functional Feeding Group (FFG) indices observed in the study area were found to be comparable to those reported from temperate and tropical regions. These indices proved effective in distinguishing ecological conditions and can serve as reliable tools for the biomonitoring and management of wadis exposed to arid climatic constraints.

2.6. Feeding Behavioral Response of Freshwater Fish to Plastic Particles with Naturally Formed Biofilm

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The pervasive plastic pollution in marine and freshwater ecosystems has led to increasingly unpredictable interactions between aquatic organisms and microplastics (particles 5‘mm), including varying instances of plastic ingestion. The incorporation of microplastics into aquatic food chains poses significant risks to health and survival of the hydrobionts, as well as potential threats to biodiversity. The ingestion of microplastics by fish can occur inadvertently or deliberately, whether plastic particles are recognized or not as food objects. In natural aquatic environments, microplastics are often colonized by biofilms consisting of complex microbial communities, including protozoa, algae, and fungi. We hypothesized that the biofilm present on microplastics influences the possibility of their consumption by fish, enhancing the attractiveness of these particles as food objects and providing a gustatory trap. Thus, we assessed the feeding behavior of juvenile African catfish *Clarias gariepinus* and climbing perch *Anabas testudineus* in the presence of two types of plastic particles: expanded polystyrene (EPS) and extruded polystyrene (XPS). Both fish species are omnivorous and feed at the water surface. We used positively buoyant microplastics sized from 3.0 to 4.5 mm, which were exposed for 30 days under natural conditions to allow biofilm formation. Our findings indicated that climbing perch actively reacted to biofilm-covered plastic objects by biting and chewing them, but never ingested them. In contrast, catfish frequently ingested both types of plastic pellets, although they rejected the XPS pellets after prolonged periods of up to 12 h. Based on these observations, we identified four patterns of feeding behavior in fish related to microplastic particles: refusal, testing and rejection, ingestion and rejection, and consumption. The first three behaviors

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are likely effective mechanisms for avoiding plastic consumption. Our results shed light on the species-specific behavioral traits that enable some fish to ignore visible plastic particles and successfully avoid consumption.

2.7. Linking Bacterial Colony Growth Dynamics to Indoor Environments

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Bacteria are widely distributed in indoor environments, yet their growth behavior and ecological differentiation remain insufficiently understood. As a pilot study, how nutritional richness influences the colony growth dynamics of indoor culturable bacteria was quantitatively analyzed. Whether microbial growth characteristics can serve as ecological indicators for classifying indoor environments was also explored. Samples were collected from a variety of indoor object surfaces representing different types and functional areas, and cultured on agar media with a variety of concentrations of LB to simulate nutrient gradients. The growth of culturable bacteria was quantitatively evaluated using multiple parameters, followed by clustering analyses based on nutrient-response profiles and growth dynamics. Distinct grouping patterns of bacterial growth behavior were observed, suggesting that variations in environmental conditions, surface materials, and possibly human-associated factors shape microbial growth strategies across indoor surfaces. Although the overall trends were stable across nutrient levels, the differentiation among clusters implied varying adaptive capacities of bacterial populations under nutrient limitation or enrichment. These findings highlight that microbial growth dynamics can reflect underlying ecological and physicochemical conditions of the indoor environment. This study provides a preliminary but quantitative framework for linking microbial growth to environmental characteristics. It indicates that bacterial proliferation potential may serve as an indicator for assessing indoor ecological states, surface susceptibility to contamination, and overall microbial resilience within human-occupied environments.

2.8. Reproductive Fitness and Population Structure of the Endangered Timber-Yielding Tree Species Phoebe bootanica (Lauraceae) in Natural Populations of Northeast India

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Phoebe bootanica (Meisn.) M. Gangop., an endemic evergreen tree species of the Indo-Myanmar biodiversity hotspot, faces significant threats due to overexploitation and habitat fragmentation, necessitating urgent conservation efforts. This study investigates the population structure and regeneration status of *P. bootanica* across its natural distribution in North-eastern India to inform conservation strategies under the projected climate change. Sporadic field surveys conducted from April 2021 to October 2023 across five states employed purposive cum random sampling in 0.1-hectare plots to assess morphological traits (height, girth, basal area, clear bole height, and bark thickness) and density of *P. bootanica* and associated tree species. Regeneration status was evaluated based on seedling, sapling, and adult counts, while girth class distribution provided insights into population dynamics; both indicate the reproductive fitness of the species in natural populations. The *Kruskal–Wallis* test revealed significant variations in morphological traits across sites, with Sorbung exhibiting the highest mean girth (357.08 $\pm$ 197.63 cm) and basal area (13.00 $\pm$ 11.59 m^2/ha). Regeneration was generally poor, with most sites showing limited evidence of seedlings and saplings, indicating challenges in natural recruitment,

possibly due to the species' recalcitrant seeds and shade-dependent regeneration. High timber demand has fragmented populations, contributing to its endangered status (IUCN B2ab(iii)). This study underscores the need for targeted conservation, including in situ and ex situ strategies, to enhance genetic diversity and population resilience. Our findings provide a baseline for long-term monitoring and sustainable management of the species, emphasizing the integration of community-level conservation initiatives to safeguard this ecologically and commercially significant species. Ecological Niche Modelling (ENM) is recommended to identify suitable habitats for future conservation planning in the projected climate change.

3. Conservation Biology

3.1. Comparative Analysis of the Trophic Ecology of the Genus Lycalopex: Patterns and State of Knowledge

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The South American canid genus *Lycalopex* comprises six species morphologically similar but inhabiting diverse environments. Although their diets have been studied locally, a comprehensive synthesis is lacking. We conducted a systematic literature review (Web of Science, Scopus, Google Scholar) to compile published trophic data, resulting in 83 studies. *Lycalopex culpaeus* was the most studied species, and Chile had the highest research output; however, rarefaction analyses confirmed this distribution reflects a significant sampling bias among species and countries. Mammals, particularly rodents, were the primary prey across the genus. We found notable trophic niche variation: *L. culpaeus* and *L. gymnocercus* displayed the greatest trophic breadth (generalists), whereas *L. griseus* was more specialized. A stronger tendency towards carnivory was observed in *L. fulvipes*, and towards herbivory in *L. sechurae*. Crucially, these latter two species were the least studied, with minimal sampling effort (600 h across only two sites), suggesting their current dietary profiles may be unrepresentative. This work provides the first integrative analysis of *Lycalopex* trophic ecology. Our findings not only reveal key dietary patterns but, more importantly, highlight critical taxonomic and geographic research biases, offering a data-driven framework to prioritize future studies on the understudied *L. fulvipes* and *L. sechurae* to inform their conservation.

3.2. Assessing Insect Pollinator Assemblages of Salvia rosmarinus in Western Himalayan Farmland

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Rosemary (*Salvia rosmarinus*) is a fragrant, medicinal, and economically valuable herb that also plays a vital ecological role in supporting pollinators. Due to its protandrous floral nature, cross-pollination by insect vectors is essential for successful reproduction, fruit set, and seed formation. To investigate its pollination ecology, a study on the insect pollinator assemblage of *S. rosmarinus* was conducted during its peak flowering season, from mid-

March to April, on the farms of India Glycols Ltd., Ghimtoli, Rudraprayag, Uttarakhand, located in the mid-altitudinal region of the Western Himalaya. Field observations were carried out between 09:00 and 14:00 h, during which a total of 28 insect pollinator species were recorded. These species belonged to three insect orders—Lepidoptera, Hymenoptera, and Diptera—representing eight families. Lepidoptera formed the most dominant group, accounting for 64% of total visitors, followed by Hymenoptera (22%) and Diptera (14%). Among families, Nymphalidae (28.6%) and Lycaenidae (21.4%) were the most represented. Prominent and frequent visitors included *Papilio machaon*, *Anthophora* sp., *Apis cerana*, *Apis mellifera*, and *Episyrphus balteatus*. Notably, several pollinator species of conservation concern were also documented, including taxa listed under Schedule II of the Wildlife Protection Act, 1972. The findings emphasize the ecological importance of *S. rosmarinus* as a pollinator-attractive plant that supports diverse insect communities. Encouraging the cultivation of rosemary in agroecosystems can promote pollinator conservation, enhance ecological balance, and contribute to both environmental and economic sustainability in the Western Himalayan farmlands.

3.3. Baboons at the Crossroads: Comparative Perspectives on Geography, Governance, and Adaptation in Africa and Saudi Arabia

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Baboons inhabit complex ecological and socio-political landscapes, from the savannahs, forests, and mountains of Africa to the arid, fragmented habitats of the Arabian Peninsula. These regions represent a crossroads where geography, governance, and adaptation intersect to shape intricate human–baboon dynamics. This integrative study synthesizes comparative insights across diverse species and management systems to reveal how spatial variation, policy frameworks, and behavioral plasticity jointly influence conflict patterns and conservation outcomes. Geography defines habitat heterogeneity and species distribution, influencing baboon ecological niches and interaction frequencies with human communities. Governance regimes range from decentralized, community-based management prevalent in African contexts to centralized, technology-driven approaches exemplified in Saudi Arabia, each mediating conflict mitigation and policy implementation differently. Adaptation, through behavioral flexibility and population responses, enables baboons to navigate increasingly anthropogenic landscapes, affecting coexistence potential and management challenges. Recognizing the convergence of these three pillars provides a multidimensional understanding essential for designing adaptive, locally tailored, and scalable conservation strategies. This framework highlights the importance of contextualizing human–wildlife interactions at ecological and governance crossroads to foster coexistence under accelerating environmental and social change. Our synthesis underscores priority areas for future research, particularly incorporating stakeholder perspectives, technological innovation, and climate resilience. Ultimately, this study offers a blueprint for reconciling ecological realities with socio-political complexities, advancing global baboon conservation in a dynamic Anthropocene.

3.4. Conservation at Risk: Ignoring Climate, Land Use, Trade, and Pesticide Threats May Cause Extinction of Amphibians in Bangladesh

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Climate change, the defining challenge of this century, is profoundly reshaping ecosystems, intensifying biodiversity loss, and driving range shifts and species extinctions. Bangladesh, with its subtropical monsoon climate and rich biodiversity, supports numerous amphibian species, of which 20.41% are currently threatened. Amphibians are particularly vulnerable due to their ectothermic physiology, complex life cycles, permeable skin, and unprotected eggs, making them highly sensitive to temperature fluctuations, habitat alteration, pesticide exposure, and overexploitation. However, few studies have simultaneously examined climate and anthropogenic pressures on these species.

This study assessed the potential impacts of climate change on ten threatened amphibian species by developing Species Distribution Models (SDMs) that incorporated climatic, land-use, pesticide, and wildlife trade data. Using 100 georeferenced occurrence records and 26 environmental predictors (19 bioclimatic and 7 ecological), I modelled current and projected (2080) suitable habitats in R with the bioclim function.

The models predict a drastic contraction of suitable habitats by 2080, placing nine species at imminent risk of local extinction, with only one species (Kaloula taprobanica) retaining 18.67% of its current range. Presently, 50% of the species occur in the Eastern Hills and Hill Tracts, while other regions host 1–2 species in fragmented patches. Spatial analysis of 49 terrestrial protected areas (9699 km^2) revealed that most suitable habitats lie outside protected boundaries; only K. taprobanica maintains significant overlap (1833 km^2). Land-use change analysis showed a reduction in forest cover to 13% by 2019, coupled with cropland and settlement expansion. Pesticide use rose from 0.21 kg/ha (1990s) to 1.1 kg/ha (2020s), posing additional chemical threats. Amphibian trade collapsed post-1990, revealing major monitoring gaps.

These findings underscore the urgent need for proactive conservation—expanding protected areas into future refugia and enforcing stricter regulations on pesticide use, land conversion, and wildlife trade—to safeguard Bangladesh's amphibian biodiversity under changing climates.

3.5. Does Floral Resource Utilization Shape the Dynamics of the Pierid Butterfly Community Across the Eastern Himalayan Landscape of West Bengal, India?

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Abundance and distribution of butterflies within a particular habitat signify floral diversity and, in turn, habitat quality. The generalized pattern of lepidopteran host plant consortium is the occurrence of host plant specialization along with a propensity of clades to remain associated with either the genus or family of host plants. The diversity of nectar plants they visit contributes towards the establishment of an elaborate plant–butterfly community. The present study was designed to explore the presence of pierid butterflies across 11 altitudinal gradients ($\leq$300 masl; 301–600 masl; 601–900 masl; 901–1200 masl; 1201–1500 masl; 1501–1800 masl; 1801–2100 masl; 2101–2400 masl; 2401–2700 masl; 2701–3000 masl; $\geq$3001 masl) in the Eastern Himalayan landscape of West Bengal, India. Identification of larval plant species followed by the determination of host plant specificity, diet breadth, and polyphagy index was undertaken. Adult nectaring resources were documented, and the information content of flower–butterfly interactions was quantified using the Shannon–Wiener Function and the Brillouin Index. The maximum number of individuals was recorded from $\leq$300 masl (791) while the minimum number of individuals was observed at $\geq$3001 masl (137). The species diversity curve showed an increasing

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trend from lowest elevation, reaching its peak at 601–900 masl (4.99) and then declined at ≥3001 masl (3.33). The highest species richness was noted at 601–900 masl (4.846) compared to the lowest value at 2401–2700 masl (1.92). A total of 56 plant species served as larval food resources for the butterflies, with 38.09% feeding from a single plant family and 35.71% from a single genus of food plant. Pierinae possessed higher diet breadth (0.435) as compared to Coliadinae (0.231). *Eurema hecabe* was most polyphagous (PI = 4.243), while 7.14% of the species were monophagous. Seventeen plant species constituted the nectar resource of the butterflies. Higher values of information content as indicated by the Shannon–Wiener Function and Brillouin Index for *Tabernæmontana coronaria* (H′ = 5.537 Ĥ = 5.267) and *Fragaria nubicola* (H′ = 5.247 Ĥ = 5.011) indicated their greater preference among butterflies.

3.6. Ecological Feasibility of Reintroducing Small Wild Cats (Leopardus guttulus and L. wiedii) on Santa Catarina Island, Southern Brazil

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The dispersal capacity of small wild cats is essential for maintaining functional populations in fragmented landscapes. However, when local extinctions occur, particularly in insular systems surrounded by densely populated continental regions, the loss of mesopredators can trigger complex ecological cascades that restructure communities and favor generalist or invasive species. Santa Catarina Island, in southern Brazil, represents one such case in which the disappearance of small wild cats has coincided with the dominance of opportunistic native species and the expansion of domestic and exotic fauna, including the invasive black-tufted marmoset (*Callithrix penicillata*). To evaluate the ecological feasibility of reintroducing small wild cats, this study integrated habitat suitability analysis, potential prey population size and biomass estimation, and camera trap monitoring within the Meiembipe Municipal Wildlife Refuge (REVIS Meiembipe), a municipal protected area on the Island. Secondary data from Projeto Fauna Floripa (2019–2021), based on island-wide camera-trap surveys, and complementary field records collected between 2024 and 2025, were combined to assess environmental suitability and identify potential release sites. Camera-trap data indicated low domestic-animal presence (20% of sampling sites) and high potential prey biomass (>167,000 kg), based on capture–mark–recapture studies and literature-based density estimates. Expert consultations (n = 11) suggested the feasibility of reintroducing *Leopardus guttulus* and *L. wiedii*. Recommendations included pre-release rehabilitation; phased soft releases of individuals meeting genetic, behavioral, and health criteria; continuous monitoring of species composition—particularly the current dominant species—and consideration of historical records confirming the former presence of both felids on the island; and telemetry-based post-release monitoring. These findings suggest the ecological viability of Santa Catarina Island for reintroduction and highlight the region's role in safeguarding small felid populations in the Atlantic Forest, emphasizing the island's unique capacity as a genetic refuge for *L. guttulus*, which is threatened on the mainland by hybridization with *L. geoffroyi*.

3.7. First Verified Dugong (Dugong dugon) Sighting at Pulau Pannikiang, with Dugong Encounters Data Across South Sulawesi's West Coastal Frontier

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In this study, we report the first scientifically confirmed sighting of *Dugong dugon* at Pulau Pannikiang (−4.3519°, 119.5994°), a mangrove-dominated island on the west coast of South Sulawesi, Barru Regency, Indonesia. The solitary individual was observed displaying foraging behavior in a *Thalassia hemprichii* seagrass meadow, followed by a school of Golden Travellay fish, *Gnathanodon speciosus.* This sighting was photographically documented in the form of photographs and a 79-s video. This record expands the known geographic range of this IUCN Vulnerable species in South Sulawesi and underscores the ecological importance of understudied coastal habitats. Interviews with fishermen revealed sporadic historical sightings, suggesting long-term site relevance. A juvenile stranding incident in August 2021, likely due to vessel trauma, emphasizes the mounting anthropogenic pressures. We contextualize this finding by compiling 13 confirmed regional records of *D. dugon* between 2007 and 2024. The compiled data, spanning 17 years, indicates a persistent yet spatially scattered presence of *Dugong dugon* along the coastal and island regions of South Sulawesi, Indonesia. Sightings, strandings, and foraging behaviors have been documented across multiple data types—ranging from peer-reviewed journals and conservation reports to local news and community observations—underscoring both scientific and anecdotal acknowledgment of dugong activity. This observation provides essential baseline data for dugong conservation and highlights the urgent need for improved habitat protection, marine mammal monitoring frameworks, and spatial planning. Strengthening local conservation policies and engaging coastal communities are crucial steps toward safeguarding seagrass ecosystems and their megafauna.

3.8. Fragile Peaks, Fading Orchids: Multiscale Patterns of Diversity, Climate Risk and Conservation in Western Himalaya

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The Himalayan canopy orchids constitute an ecologically significant yet understudied group of epiphytic plants that thrive in the fragile montane ecosystems of the North Western Himalaya. This study examines the patterns of diversity, distribution, and climate sensitivity of canopy orchids along altitudinal gradients, integrating multiscale field data, GIS-based habitat mapping, and species distribution modelling. Results revealed a distinct mid-elevation diversity peak (1800–2200 m) driven by microclimatic stability and host-tree diversity, with projected models showing a 23–41% contraction of suitable habitats and potential loss of unique evolutionary lineages under high-emission scenarios.

By combining landscape-level environmental data with fine-scale ecological observations, this research highlights temperature seasonality and canopy humidity as dominant predictors of orchid occurrence and demonstrates how microhabitat heterogeneity buffers orchids from macroclimatic changes. Projected models indicated a significant upward contraction of suitable habitats by 23–41% under high-emission scenarios, particularly

threatening narrowly distributed taxa such as *Dendrobium* sp. and *Bulbophyllum* sp. The findings underscore the vulnerability of canopy-dependent orchids to both anthropogenic pressures and climate-induced habitat fragmentation. Conservation efforts should prioritize the protection of mid-elevation forest canopies and the creation of climate-resilient corridors to facilitate species persistence. Overall, this work provides a baseline for long-term monitoring and adaptive management strategies essential for safeguarding Himalayan orchid diversity under future climate change scenarios.

3.9. Fungal Diversity and Community Structure in Soils of High-Altitude Ecosystems of Sathyamangalam, India

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The Sathyamangalam region, straddling the transitional zone between India's Western and Eastern Ghats, represents a unique, high-altitude (above 4000 feet MASL) biogeographic area critical for biodiversity. Fungi, as key drivers of decomposition, nutrient cycling, and plant health, are integral to this ecosystem's function. This study aimed to characterize the soil fungal communities across different habitats and elevations within this ecologically significant landscape. Environmental DNA (eDNA) was analyzed using fungal ITS metabarcoding, sequenced in Illumina 250 bp paired-end chemistry and the QIIME2 pipeline, with taxonomic assignments based on the robust UNITE database. Sequencing yielded a total of 417,612 quality-filtered reads across six samples studied, resulting in a high observed diversity ranging from 1047 to 1460 Operational Taxonomic Units (OTUs) per sample (Total OTUs: 7495). The communities were rich in key functional groups, including dominant saprotrophic (*Penicillium*, *Aspergillus* and *Mortierella*) and the mycorrhiza of both ectomycorrhizal and arbuscular mycorrhizal fungi. The identified species sequences are publicly available via NCBI GenBank Accession Numbers (PQ523512-PQ527698). Crucially, taxonomic annotation indicated the potential presence of new species records for India, and a notable fraction of reads identified only at the Phylum level suggests significant novel fungal diversity new to science. Many of the sequences are simply identified as they belong to Kingdom Fungi stating their distribution as Dark Taxa. Community composition varied significantly, reflecting differences between the distinct Western and Eastern Ghats ecosystems. This high-resolution inventory provides a vital baseline for the high-altitude soil mycobiota of Sathyamangalam. The observed species richness and identification of potential novel fungal diversity underscore the ecological complexity of this transitional zone and highlight its importance for fungal biogeography and conservation.

3.10. Habitat Preferences and Behavior of the Great-Tailed Grackle Quiscalus Mexicanus in Wetlands with Different Levels of Urbanization on the North Pacific Coast of Mexico

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The current global population trend is towards greater concentration in cities, which presents a potential negative risk to wetlands and the species that inhabit them, especially birds, when they are encroached upon by urban growth that destroys the vegetation used

for perching, roosting, nesting, and feeding. Bird species capable of adapting to urban environments are the ones that ensure their permanence and success over other species with less adaptive capacity. Therefore, in the present study, the abundance and distribution of the Mexican Grackle Quiscalus mexicanus were obtained in two urban wetlands with different levels of urbanization and land-use policies. The point count method was used, and the behavior of the species, types of vegetation, urban infrastructure, and human activities were also recorded. The results showed that the lowest abundance occurred in the wetland that was the most urbanized, with a land-use policy for urban development, intensive recreational use, and a preference for street lighting and sidewalks. In contrast, the highest abundance was found in the wetland with a land-use policy focused on protection, showing habitat preference for areas with trees and guided visit activities for environmental education. It can therefore be concluded that this species develops better in less urbanized environments with abundant trees.

3.11. Herons (Pelecaniformes: Ardeidae) Associated with an Artificial Water Body in Xalapa, Mexico

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Artificial water bodies located within urban areas play a crucial role in biodiversity conservation, as they act as remnants of the original ecosystems and provide alternative habitats for aquatic birds.

This study was conducted in an artificial lake located within the city of Xalapa, Mexico, known as "Lago USBI". Data collection was carried out using the fixed-radius point count method, recording all aquatic bird individuals observed within a 20-m radius for 10-min intervals. Bird identification was performed using specialized field guides, and weekly observations were conducted from February to April 2024. The relative abundance of each species was calculated, and its conservation status was classified according to the categories of the IUCN Red List.

A total of five heron species (Ardeidae) were identified, all categorized as "Least Concern" by the IUCN. The recorded species and their respective relative abundance values were as follows: *Butorides virescens* (78%), *Ardea alba* (11%), *Egretta thula* (5%), *Ardea herodias* (4%), and *Nyctanassa violacea* (2%).

Although the lake "Lago USBI" is an artificial habitat, it hosts 31% of the heron species reported for Mexico. Despite all being listed as "Least Concern," these species warrant attention in environmental conservation studies, as they are often ecologically associated with other key species, thus providing indirect protection.

The findings of this study highlight the ecological importance of artificial water bodies as alternative habitats for species in urban environments. Consequently, this research underscores the potential of such habitats as valuable sites for future studies on urban ecology and heron community dynamics.

3.12. Hunters in Danger: Evolutionary Diversity and Conservation Priorities in Carnivorous Mammals of the American Continent

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Mammals of the order Carnivora play a fundamental role in ecosystems as top predators, with some taxa designated as flagship and umbrella species. In the Americas, they have a complex biogeographical history, shaped by processes of colonization and adaptive radiation that have generated a high diversity of lineages. However, in the face of the global biodiversity crisis, conservation efforts have traditionally focused on species richness, overlooking evolutionary relationships. This limited approach prevents the recognition of areas and species with unique evolutionary value, which is crucial for planning effective strategies. In this study, we evaluated the distribution of the evolutionary diversity of carnivorous mammals in the Americas and its relationship with biological and geographical variables, using phylogenetic metrics that incorporate extinction risk and biogeography. We analyzed 104 species using phylogenies, geographic distributions, and threat categories obtained from public databases and the literature. Using this information, we calculated phylogenetic metrics of Evolutionary Distinctiveness (ED) and incorporated extinction risk (EDGE), evaluating their relationship with body size, altitude, and latitude. Additionally, we estimated Biogeographically weighted Evolutionary Distinctiveness (BED) and mapped spatial patterns of richness, metrics, and priority conservation areas. The evolutionary diversity of carnivores was unevenly distributed across regions and taxonomic groups. In terrestrial species, ED and EDGE scores decreased with altitude and followed a unimodal pattern with latitude, concentrating in the tropical belt; in marine species, values clustered in the temperate zones of the Northern Hemisphere, where a relationship between body size and ED scores was also observed. Spatial patterns of richness were consistent with those of ED and EDGE but differed markedly from those obtained with BED. These results highlight the importance of incorporating evolutionary metrics into the prioritization of American carnivores, protecting their evolutionary history and optimizing conservation planning and resource allocation.

3.13. Ignoring Climate Change May Falsify Conservation Efforts for the Threatened Reptiles of Bangladesh

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Climate change is one of the major threats facing our planet in the coming years, and its detrimental impacts on biodiversity are being documented. Numerous threatened species have already experienced population and species-level extinctions as a result of these effects. Detailed information regarding the distribution patterns, habitats, suitable space, and responsible variables that limit and shift the distribution of these threatened species is necessary for effective and targeted conservation planning and forecasting, particularly in situations where they face multiple conservation challenges. Species Distribution Models (SDMs) are frequently used to measure the correlation between the occurrence of such species and spatial variables, enabling the extrapolation of this relationship to new areas or time periods. Twelve bioclimatic and seven environmental variables from 100 occurrence points of 37 threatened reptiles of Bangladesh are used to train the Bioclim model under current climatic conditions. The model is then projected into the 2080 climatic scenario to illustrate how collective and individual species are expected to respond to future climate change under various assumptions. Out of the 37 species of threatened

reptiles, 10 species (*Batagur baska*, *Chelonia mydas*, *Lepidochelys olivacea*, *Dermochelys coriacea*, *Dopasia gracilis*, *Lygosoma punctata*, *Rhabdophis himalayanus*, *Boiga siamensis*, *Crocodylus porosus*, and *Heosemys depressa*) are predicted to lose 100% of their suitable climatic space by the year 2080. Elevation and bio-ecological subregions play pivotal roles in shaping and constraining the distribution of these reptiles due to their impact on habitat suitability and climatic conditions. Proactive conservation strategies, such as the establishment and expansion of protected areas within their climatically appropriate space, should be taken into consideration in order to preserve these imperiled species.

3.14. Impacts of Habitat Fragmentation on Pollinator Diversity in Agricultural Landscapes

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Habitat fragmentation poses a significant threat to pollinator communities, particularly within intensively managed agricultural landscapes. In India, the expansion of monoculture cropping and urban encroachment has led to the progressive isolation of semi-natural habitats that traditionally support a high diversity of pollinators. This study examines the relationship between habitat configuration, landscape heterogeneity, and pollinator diversity across multiple agroecosystems in northern and central India. Field surveys and spatial analyses were employed to quantify pollinator abundance, species richness, and community composition in relation to patch size, edge density, and habitat connectivity. Results indicate a marked decline in both the taxonomic and functional diversity of pollinators with increasing habitat isolation and reduced floral resource availability. Fragmented landscapes exhibited a dominance of generalist species such as Apis cerana and A. dorsata, while specialist and solitary bees showed sharp declines in occurrence. The analysis further suggests that small fragments with high structural complexity such as hedgerows, fallow fields, and riparian buffers play a disproportionately important role in sustaining pollinator assemblages. These findings highlight the urgent need to integrate biodiversity-friendly land-use planning within agricultural policy frameworks. Enhancing habitat connectivity through agroforestry corridors, diversified crop mosaics, and conservation of native vegetation can mitigate the negative effects of fragmentation. Sustaining pollinator diversity is not only critical for maintaining ecosystem resilience but also for ensuring stable crop yields and food security in India's agrarian landscapes.

3.15. Long-Term Monitoring Efficiency and Identification Success in the Reintroduction of the Vinaceous-Breasted Amazon (Amazona vinacea)

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The vinaceous-breasted amazon (*Amazona vinacea*), one of the most threatened Neotropical parrots, has been the focus of a long-term reintroduction project at Araucárias National Park, Brazil. Between January 2011 and June 2023, twelve release events were conducted, followed by periodic monitoring using a combination of methods: field records by the technical team, citizen-scientist reports and camera traps. Monitoring by the technical team relied on visual inspection and vocalization records to locate and identify individuals.

Radio telemetry was used when resources were available and for as long as transmitter batteries remained active. Monitoring frequency increased between 2017 and 2023, driven by an increase in financial resources, expanded field effort by the technical team and stronger involvement of local communities. A total of 8671 monitoring records and reports were analyzed. The contribution of citizen scientists increased from less than 5% of records before 2016 to over 25% after 2019, highlighting the growing role of participatory monitoring in data collection across broader spatial and temporal scales. The introduction of numbered medal tags in 2019 enhanced the ability to recognize individual parrots, raising identification success from 34–73% to over 90%. These findings demonstrate the refinement of field methodologies and the benefits of integrating professional and community-based monitoring. Post-release monitoring is fundamental for assessing survival, adaptation and reproduction, providing feedback for project adjustments. Despite notable advances, reliably finding and identifying translocated individuals from a long distance in the Atlantic Forest remains one of the project's greatest challenges. The adoption of emerging technologies, such as drone-assisted telemetry, small and parrot resistant GPS devices, and AI-based image and sound recognition, remains largely limited by costs and technical availability but represents a crucial future direction for improving long-term monitoring of *A. vinacea* in the wild.

3.16. Short-Term Semen Storage of the Bulatmai Barbel (Luciobarbus capito) for Conservation Programs

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In aquaculture, short-term sperm storage is crucial for selective breeding programs since it helps synchronize gamete availability, enables controlled fertilization, and aids in genetic management and conservation efforts. In the present study, the percentage of motile sperm (MP) and motility time (MT) in the Bulatmai barbel (*Luciobarbus capito*) were evaluated after chilled storage for 0, 24, 48, 72, and 96 h at 4 °C. Samples were collected from five mature males with an average weight of 1910.0 ± 420.4 g. Before any manipulation, fish were anesthetized using clove powder extract. Sperm was collected using a sterile syringe by applying gentle pressure to the abdominal region. Each sperm sample (0.5 mL) was stored in plastic tubes at 4 °C. The results indicated significant differences in MP at all intervals after chilled storage compared to the initial sampling (P 0.05). A considerable reduction in the MT was observed starting at 72 h after chilled storage compared to the initial time (P 0.05). All samples remained active for up to 48 h following storage. The results indicated that sperm quality significantly declines even after 24 h in chilled storage (with a 24% reduction in active spermatozoa). Future studies should investigate various storage media to assess their potential for extending the duration of sperm preservation under specific storage conditions. Considering the significance of broodstock management, it is recommended to limit the storage of Bulatmai barbel sperm samples to a maximum of 24 h for controlled reproduction purposes.

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4. Infection Biology

4.1. Fasciola Worm-Based Antigen and Its Potentials in the Diagnosis of Urogenital Schistosomiasis in Resource-Limited Endemic Areas

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Urogenital schistosomiasis, caused by the flatworm-*Schistosoma haematobium*, is a neglected tropical disease ranked second to malaria in terms of its socio-economic and public health importance in tropical and subtropical areas. Early identification and diagnosis are crucial for effective disease management and control. This study aimed to assess the immunodiagnostic potential of crude *Fasciola gigantica*-worm antigen (FWA) as a target for detecting anti-*S. haematobium* antibodies in sera and urine samples. An indirect ELISA was used to assess antibodies against these antigens in serum and urine samples from *S. haematobium*-infected and non-infected individuals (n = 48) in schistosomiasis endemic (NE) and non-endemic (NNE) areas, with microscopy as diagnostic reference. The Area under the receiver operating characteristic (ROC) curve (AUC), sensitivity (SS), and specificity (SP) of FWA for sera in diagnosing *S. haematobium*-infected individuals using ELISA were 0.957, 97.92%, and 70.82% for positive and NNE samples and 0.542, 70.83%, and 31.25% for infected and NE samples, respectively. Similarly, the diagnostic performances of FWA measured by AUC, SS, and SP were 0.9679, 97.92%, and 83.33% for positive and NNE samples and 0.9670, 97.92%, and 85.33% for positive and NE urine samples, respectively. *S. haematobium*-specific antibody production against FWA-sera and urine was significantly higher in infected individuals than in non-infected groups in endemic and non-endemic areas (P 0.0001). This study demonstrated the feasibility of serological diagnosis of urogenital schistosomiasis with *Fasciola gigantica* worm antigens in resource-constraint where schistosomiasis is endemic. The urine samples exhibited better diagnostic metrics than the sera samples, which can be leveraged as a non-invasive biological sample for diagnostic advantage.

4.2. Assessment of Microplastic Translocation in Human Blood and Placental Tissue Using Raman Spectroscopy and FTIR Imaging: Implications for Fetal–Maternal Health

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Microplastic (MP) contamination in the human body is an emerging concern in biomedical science, particularly due to recent evidence indicating their translocation into systemic circulation and placental tissue. This literature review evaluates current research on the presence of MPs in human blood and placenta, highlighting the clinical implications and the efficacy of two advanced spectroscopic techniques, Raman spectroscopy and Fourier-transform infrared (FTIR) imaging, for in situ detection. Research published between 2015 and 2024 was systematically reviewed using MEDLINE and ScienceDirect, focusing on observational human studies that employed spectroscopic MP detection. The search strategy incorporated keywords such as "microplastic translocation," "human blood," "placenta," "Raman spectroscopy," "FTIR imaging," and "fetal-maternal health." Microplastics were detected in 88.9% of analyzed blood samples, with polystyrene, polypropylene, and

polyethylene being the predominant polymers. Placental tissues showed a median of 12 MP particles per 10 g, mainly localized in the chorionic villi and maternal decidua. Raman spectroscopy demonstrated superior sensitivity for detecting smaller MPs, especially nonpolar polymers in digested samples, while FTIR was more effective for larger particles in whole tissue sections. Clinically, MP presence was associated with elevated inflammatory markers such as C-reactive protein and interleukin-6, prolonged activated partial thromboplastin time, and increased fetal liver enzymes, suggesting systemic inflammation, immune modulation, hepatocellular stress, and potential impairment of placental function. The findings underscore the biomedical urgency of understanding MP exposure pathways and their effects on fetal–maternal health. To advance diagnostic precision and maternal–fetal care, it is recommended that future biomedical research integrates standardized MP detection protocols into clinical toxicology workflows and explores targeted molecular biomarkers for early identification and risk stratification of MP-induced placental dysfunction.

4.3. Heterologous, Overlapping, Differentially Expressed Isg-Signatures Reveal Shared Patterns Across Autoimmune Diseases

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Type I interferon (IFN) signalling is a key axis implicated in multiple autoimmune diseases, driving chronic immune dysregulation. While disease-specific interferon-stimulated gene (ISG) signatures have been studied extensively, the presence of a universal ISG signature across all autoimmune diseases remains unproven. Here, I perform a **pan-disease comparative transcriptomic analysis** to identify *heterologous* ISG overlaps—i.e., ISGs that are shared across subsets (pairs or triplets) of diseases, rather than across all.

I selected publicly available gene expression datasets for autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and primary Sjögren's syndrome. Using standardized R workflows (e.g., limma), I identified differentially expressed genes for each disease ($|\mathbf{log_2FC}| > 1$, **adj.** p **0.05**). These DEGs were intersected with a curated ISG reference catalog to isolate disease-specific ISG sets. I then performed pairwise and triple-wise overlap analyses to detect shared ISG signatures across disease combinations. Enrichment analyses (Gene Ontology, KEGG) elucidated the functional context of shared vs. unique ISG modules, and network-based visualization characterized hub genes and interconnections.

I found **no ISG** that is commonly differentially expressed across all autoimmune diseases studied. However, multiple ISGs are shared in subsets, e.g., between SLE and pSS, RA and MS, and in some cases trios such as pSS–RA–SLE. Overlapping ISG modules are enriched in antiviral defence, cytokine signalling, and JAK–STAT activation, whereas disease-unique ISGs map to pathways involving tissue remodelling, B-cell signalling, and metabolic processes. Network analysis identifies hub ISGs within overlapping modules that may serve as cross-disease regulatory nodes.

These results support a model in which **autoimmune disorders share *heterologous* rather than universal interferon signatures**. Overlapping ISG modules across disease subsets point toward convergent immune dysregulation, while unique ISGs may contribute to disease-specific pathology. The hub ISGs uncovered are promising candidates for cross-disease biomarker development or targeted therapy.

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4.4. Development and Accuracy Determination of a Peptide-Based Diagnostic Test for SARS-CoV-2 Based on the N-Terminal Ectodomain of the Membrane Glycoprotein

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Background: The N-terminal ectodomain (NTE) of the SARS-CoV-2 membrane (M) glycoprotein is a short, flexible region that remains exposed on the virion surface. Despite its small size and conformational plasticity, this region contains conserved linear epitopes that may serve as practical surrogates for full-length proteins in serological diagnostics.

Objective: The objective of this study was to develop and evaluate a synthetic peptide-based diagnostic assay targeting the NTE of the SARS-CoV-2 M protein.

Methods: Peptide analogs of SARS-CoV-2 M NTE were designed from a cluster of epitopes retrieved on February 2021 from the Immune Epitope Database (IEDB). M1 (CADSNGTITVEELKKLLEQC-am) is a polymerizable peptide used as coating antigen in a diagnostic indirect ELISA, while anti-M1 is an antipeptide antibody affinity-purified from the sera of rabbits immunized with M1i (MADSNGTITVEELKKLLEQC-am). The binding affinity of monomeric and polymeric M1 was estimated in terms of the dissociation constant (K_d), taken from the slope of ELISA data performed against anti-M1, and linearized using the Liliom method. To determine diagnostic accuracy, M1-based indirect ELISA was performed on 221 biobanked plasma controls and on 1222 serum samples corresponding to 544 RT-PCR–confirmed adult patients admitted to the Philippine General Hospital, a 1335-bed tertiary referral center, during October 2020–February 2021, before SARS-CoV-2 vaccines became available locally.

Results: Polymeric M1 exhibited a two-fold gain in apparent affinity (K_d = 4.33 nM) compared with the monomeric form (K_d = 8.00 nM). Clinical validation of the M1-based diagnostic ELISA yielded a post hoc (Youden J) sensitivity of 95.26% and specificity of 52.27%, with an overall diagnostic accuracy of 88.70%.

Conclusion: M1 demonstrates that synthetic peptide antigens can serve as stable, high-sensitivity antigens for diagnostic assays. This design principle may be applied to other emerging pathogens where rapid assay development and scalability are critical.

4.5. Oral Mucoadhesive Newcastle Disease Vaccine: Development, Characterization, and Clinicopathological Evaluation in Challenged Chickens

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Effective vaccine delivery is important for sustainable poultry health management, especially in regions facing infrastructural and resource limitations. Natural polymers such as cashew tree gum (CTG) is a promising, low-cost, biodegradable material for oral

vaccine delivery, yet its veterinary applications is underexplored. This research assessed CTG's suitability as a vaccine carrier, its formulation into mucoadhesive microbeads, and its practical value in improving Newcastle Disease (ND) control in poultry production in Nigeria.

CTG was harvested and purified for physicochemical and phytochemical characterization. Broilers and field flocks were then investigated to evaluate CTG-alginate-based mucoadhesive vaccine formulations and immune performance. Hemagglutination inhibition (HI) assays, hematological parameters, heterophil-to-lymphocyte (H:L) ratios, and histopathology of lymphoid organs were assessed. A field survey across six southwestern states also analyzed vaccination practices and risk factors for ND outbreaks.

Purified CTG showed high yield (81.6%), near-neutral pH (4.08), and absence of toxic metals, confirming its safety and biocompatibility. In the field, 63.7% of farms recorded sub-protective antibody titres, largely due to poor biosecurity, vaccination and cold-chain lapses. Experimentally, CTG-alginate microbead vaccines produced significantly higher and more durable antibody titres (GMT $\geq$ 203.2; P 0.05), improved lymphoid activity, and lower H:L ratios compared with conventional aqueous vaccines. The local LaSota strain delivered via mucoadhesive beads induced the most persistent protection without affecting growth.

Developing CTG-based vaccine microbeads can significantly enhance vaccine efficacy by prolonging mucosal antigen retention and exposure, resulting in stronger and sustained antibody responses. This approach is cheaper, user-friendly, and practical for poultry farmers in settings with financial, infrastructural, or cold-chain constraints. Collectively, it represents a control package suitable to the production and socio-economic context of backyard poultry farmers in Nigeria and similar regions.

4.6. Targeting Infection: From Molecular Pathways to Novel Therapeutic Strategies

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Infectious diseases remain a major global health challenge, causing significant morbidity and mortality. Beyond antimicrobial resistance, challenges in infection management include the immense diversity of pathogens, their ability to persist in latent forms, and the complexity of host–pathogen interactions that allow pathogens to evade immune defences. The ability of pathogens to invade, colonize, and manipulate host systems is governed by complex molecular mechanisms that are often finely tuned to evade host defences. In this study, we examined key molecular pathways involved in host–pathogen interactions, focusing on factors that contribute to pathogen survival, replication, and immune evasion. Using a combination of cellular models, molecular assays, and biochemical analyses, we identified critical virulence factors and host signalling pathways that represent potential intervention points. Structure–activity relationship (SAR) analyses and computer aided drug design (CADD)-based approaches were employed to identify molecular features crucial for pathogen survival and for designing targeted therapeutics. We discuss conventional approaches, such as antibiotics and antiviral drugs, alongside rational, host-directed strategies that modulate immune responses to improve treatment outcomes. Recent developments in antimicrobial therapy, including small-molecule inhibitors, antimicrobial peptides, and biologics, are highlighted, with a perspective on emerging technologies. By integrating mechanistic understanding with translational approaches, this work highlights the importance of a multilevel perspective on infection biology, encompassing molecular,

cellular, and organismal responses. These findings provide a context for designing precise and effective interventions, offering potential pathways for improved management of infectious diseases and reduced treatment-related side effects.

4.7. Antimicrobial Resistance and Virulence of Aeromonas Isolates from Endangered Schizothoracinae

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Introduction: Emerging pathogenic bacteria represent a growing threat to aquaculture, particularly for rare and protected fish species. Populations of Schizothoracinae have experienced declines due to environmental degradation, habitat loss, and anthropogenic pressures. Despite their ecological and economic importance, microbial threats to these fish remain poorly understood. Understanding the pathogenic potential of bacterial species in Schizothoracinae is essential for developing effective biosecurity measures and conservation strategies. This study aimed to investigate the occurrence, antimicrobial resistance, biofilm-forming capacity, and potential virulence of *Aeromonas* associated with Schizothoracinae, providing baseline data for disease management and aquaculture safety.

Methods: *Aeromonas* was isolated from multiple organs of Schizothoracinae and characterized using Gram staining, standard biochemical tests, and molecular identification methods. The isolates were evaluated for antimicrobial susceptibility and biofilm formation, while in silico genomic analyses of related strains were conducted to predict the presence of virulence and resistance genes.

Results: All isolates were confirmed as *Aeromonas*. They displayed multidrug resistance and variable biofilm-forming ability, with stronger biofilm producers correlating with higher antimicrobial resistance. Genomic analysis suggested the presence of genes linked to virulence and antibiotic resistance, indicating horizontal gene transfer as a likely mechanism driving adaptability and pathogenic potential.

Conclusions: This study provides the first evidence of *Aeromonas* as a potential pathogen in Schizothoracinae, highlighting its pathogenic and zoonotic potential. The findings emphasize the importance of monitoring microbial threats in protected fish species, supporting aquaculture biosecurity, conservation breeding programs, and future research include.

4.8. Bioactive Profiling of Citrus aurantium Peel Ethanol Extract and Effects on Escherichia coli and Staphylococcus aureus Drug Target Proteins

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The rising threat of antimicrobial resistance necessitates the search for novel bioactive molecules from natural sources. This study investigated the phytochemical composition, antibacterial potency, and molecular docking interactions of *Citrus aurantium*

peel ethanol extract against *Escherichia coli* outer membrane and topoisomerase proteins and *Staphylococcus aureus* toxins as drug target proteins. Qualitative and quantitative phytochemical compositions were examined using standard analytical methods, chemical compounds were evaluated and qualified using Gas Chromatography–Mass Spectrometry (GC-MS), antibacterial effects were investigated in silico and validated in vitro. Qualitative and quantitative analyses revealed high concentrations of flavonoids (4.54 ± 0.11%), alkaloids (1.6 ± 0.03%), terpenoids (1.35 ± 0.01%), tannins (1.02 ± 0.05%), phenols (0.97 ± 0.07%), and saponins (0.80 ± 0.01%). GC–MS profiling identified several dominant compounds, including β-D-glucopyranose, neo-inositol, 8-(2,3-dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one, and D-allose. In silico docking studies against bacterial druggable proteins (PDB IDs: 4C56 and 3MFG, which are *S. aureus* toxins; 1BXW, and 3FV5, which are *E. coli* outer membrane and topoisomerase proteins) revealed strong binding affinities (–6.477 to −8.774 kcal/mol), comparable to standard antibiotics. ADMET predictions confirmed favorable pharmacokinetic and safety profiles, with most lead compounds displaying high intestinal absorption, low hepatotoxicity, and compliance with Lipinski's rule of five. The extract exhibited stronger antibacterial activity, producing inhibition zones of 25.11 ± 0.017 and 23.04 ± 0.25 mm against clinical isolate of *S. aureus* and *E. coli*, respectively, at a concentration of 10 mg/mL, comparable to ciprofloxacin (30.35 ± 0.26 mm). These findings highlight C. aurantium peel phytoconstituents as promising scaffolds for antibacterial drug development and justify further in vivo validation for combating multidrug-resistant pathogens.

4.9. Characterization of Multidrug-Resistant Bacterial Pathogens from Malaria Patients in Ondo State, Southwest Nigeria, Using Molecular and Phenotypic Approaches

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Co-infection of malaria and bacterial pathogens poses a major threat to public health, particularly in regions with high malaria endemicity and limited antibiotic stewardship. This study investigated the multiple antibiotic resistance (MAR) patterns and Extended-Spectrum Beta-Lactamase (ESBL) gene distribution of bacterial isolates from the blood of malaria patients attending government hospitals in Ondo State, Nigeria. Blood samples from malaria-confirmed patients were cultured for bacterial isolation and identification using standard microbiological techniques. The antibiotic susceptibility of isolates was determined by the Kirby–Bauer disc diffusion method. Multiple antibiotic resistance phenotypes and MAR indices were calculated. ESBL production was detected phenotypically and genotypically using PCR. Plasmid extraction and curing were performed to determine the role of plasmids in resistance dissemination. Bacterial isolates exhibited high resistance to amoxicillin (96.52%), cefuroxime (89.57%), chloramphenicol (86.09%), and tetracycline (83.48%), but showed low resistance to ofloxacin (16.52%) and gentamicin (24.35%). *Enterobacter aerogens*, *Pseudomonas aeruginosa*, and *Shigella dysenteriae* displayed complete resistance (100%) to several antibiotics, including amoxicillin and chloramphenicol. A total of 34 multiple antibiotic-resistant (MAR) phenotypes were recorded, with the predominant pattern being AUG–CRX–TET–CH–S–AMX, observed in *E. coli*, *S. Typhi* and *S. enteritidis*. The most frequent MAR indices were 0.625 (22.61%) and 0.75 (21.74%).

Phenotypic detection revealed ESBL occurrence rates of 66.09%, 31.30%, and 33.91% using disc diffusion, approximation, and combination methods, respectively. Genotypically, the prevalences of bla TEM, SHV and CTX-M genes were 12.82%, 12.82%, and 33.33%, respectively, with *E. coli* and *S. Typhi* being the dominant carriers. Plasmid sizes ranged from 2 to 80 kbp, with some isolates harboring multiple plasmids. Post-curing analysis showed loss of resistance in most isolates, confirming plasmid-mediated resistance in several strains. This study highlights the alarming prevalence of multidrug-resistant and ESBL-producing bacteria among malaria patients in Ondo State, Nigeria. The detection of plasmid-mediated resistance underscores the urgent need for rational antibiotic use, continuous surveillance, and infection control strategies to mitigate antimicrobial resistance in malaria-endemic regions.

4.10. Comprehensive Workflow for Bacterial Genome Analysis: From SRA Raw Reads to Reference Mapping and Annotation

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Introduction

Bacterial genomics has been transformed by next-generation sequencing (NGS), enabling faster, large-scale, and cost-effective analyses. However, the complexity of computational tools and multi-step processing pipelines often poses difficulties for researchers with limited bioinformatics expertise. To address this, we created a modular bioinformatics pipeline optimized for Illumina bacterial genomes. The workflow combines essential steps, including quality control, trimming, read mapping, variant calling, de novo assembly, annotation, and visualization into an automated, reproducible, and user-friendly pipeline.

Methods

Raw sequencing reads of *Pseudomonas aeruginosa* strain 2025SY-00129 (SRR33893847) were retrieved from NCBI SRA using SRA-Toolkit. Quality control and trimming were performed using FastQC, MultiQC, and Trimmomatic. BWA was used to map the reads to the *Pseudomonas aeruginosa* strain-2507 reference, and FreeBayes was used to identify variations. De novo assembly was generated with SPAdes, assessed using QUAST, and annotated with Prokka. All processes were carried out using automated shell scripts in Conda environments to ensure reproducibility.

Results

The workflow produced a high-quality *Pseudomonas aeruginosa* draft genome of approximately 6.93 Mb, comprising 193 contigs, with robust assembly statistics (N50 = 298,141 bp, GC content = 66.11%, and average coverage depth = 29x) indicating high completeness. The genome annotation provided a thorough insight into the genomic architecture by identifying 6379 coding sequences, including 2767 hypothetical proteins, 68 tRNAs, one tmRNA, and one CRISPR array. The workflow also produced comprehensive outputs, including alignment summaries, variant density plots, and coverage maps, demonstrating its efficiency, strength, and reproducibility for bacterial genome analysis.

Conclusion

This modulated workflow offers a reproducible approach to bacterial genome analysis with open-source tools. It offers the generation of accurate genome assemblies and annotations, providing a solid basis for downstream analysis of bacterial genomes. The complete workflow and documentation are available at https://github.com/mdarsikdar/bacterial-upstream-analysis (accessed on 1 June 2026).

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4.11. Dot Blot Validation of Eluted Schistosoma Immunogenic Proteins for Rapid Schistosomiasis Diagnosis

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Schistosomiasis remains a major public health concern in endemic regions where rapid and accurate diagnosis is vital for effective control. Persistent diagnostic challenges arise as a result of crude parasite antigen-based assays, which often provoke non-specific immune responses and compromise accuracy. This study validated two immunoreactive proteins eluted from *Schistosoma haematobium* soluble egg antigen (SEA) and *Schistosoma mansoni* worm antigen proteins (WAPs) as improved diagnostic tools for field deployment using dot blot assays.

SDS–polyacrylamide gel electrophoresis and Western blot assays identified immunoreactive protein bands from SEA and WAP, which were then evaluated using ELISA on 40 repository samples (20 positive and 20 negative). The bulk of the validation work employed dot blot assays, which is the primary qualitative method used to demonstrate clear immunogenicity of each antigen against patient urine. Statistical analyses, including tests of mean optical density, area under ROC curve (AUC), sensitivity, and specificity, were performed using GraphPad Prism 8.0.1 and MedCalc webserver.

Two highly immunoreactive proteins were successfully eluted: 72 kDa from *Sh* SEA and 95 kDa from *Sm* WAP, with diagnostic cut-offs of 0.3383 and 0.1102, respectively. The 72 kDa protein demonstrated excellent performance with 95% sensitivity and 90% specificity, while the 95 kDa protein showed 90% sensitivity and 95% specificity. Both exhibited excellent diagnostic accuracy of 72 kDa (AUC = 0.96; 95% CI: 0.90–1.00) and 95 kDa (AUC = 0.96; 95% CI: 0.91–1.00). Dot blot validation clearly revealed the immunogenic potential of both proteins, visually differentiating positive from negative samples. ELISA results showed significant differences in mean optical density between positive (0.75 ± 0.27), (0.35 ± 0.19) and negative (0.23 ± 0.15), (0.07 ± 0.02) samples (P 0.0001).

The 72 kDa and 95 kDa eluted proteins demonstrated strong immunogenicity and diagnostic precision, addressing the limitations of crude antigen-based assays and offering more specific and reliable tools for schistosomiasis detection in endemic regions.

4.12. Entomopathogenic Fungi as Biological Regulators of Crop Pests: Integrating Molecular Mechanisms and Ecological Applications

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Metarhizium anisopliae and *Beauveria bassiana* function as natural enemies that control various insect pests through their entomopathogenic properties. The biological control method based on these fungi provides an environmentally friendly alternative to chemical pesticides. The success of these fungi depends on multiple stages that make up their infection process. The infection process starts with spore attachment to the cuticle, followed by penetration and successful evasion of the host immune system. The scientific community needs to understand both molecular and ecological aspects of these fungi to enhance their

performance in different environmental settings. This research compiles current knowledge about entomopathogenic fungi from 2015 to 2025 regarding their biological characteristics and practical applications. Research into genomic and transcriptomic data has discovered multiple virulence-related genes and stress-tolerant antioxidant metabolites that help fungi adapt to their hosts. Research conducted in controlled environments and outdoor fields demonstrates that these fungi perform better when scientists use enhanced formulations, resistant plant strains, and beneficial microbial associations in the soil and plant tissues. The combination of molecular research with ecological methods will enhance the potential of entomopathogenic fungi for future pest control methods. Biological control will become a reliable and sustainable agricultural tool when scientists develop new formulation methods and study how plants and fungi interact with each other and how they work together for better results.

4.13. Evolution of Antimicrobial Resistance and Virulence in Avian Pathogenic Escherichia coli: A Dual Threat to Animal and Human Health

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Avian colibacillosis, a pervasive bacterial infection in poultry, is caused by avian pathogenic *Escherichia coli* (APEC). The pathogenicity of these strains is primarily associated with specific virulence factors such as serum resistance and α-hemolysin production. The uncontrolled and excessive use of antibiotics in poultry production has greatly contributed to the emergence of resistant and even multidrug-resistant (MDR) APEC strains.

In the present study, fifty APEC isolates were characterized for their virulence and antimicrobial resistance traits. Almost all isolates exhibited serum resistance and produced α-hemolysin, while 42% were Congo red positive, indicating the presence of virulence-associated phenotypes. Antimicrobial susceptibility testing revealed alarming resistance rates: 86% to tetracycline, 76% to nalidixic acid, and 60% to amoxicillin. Conversely, tobramycin and imipenem remained largely effective against most isolates.

However, colistin resistance was detected in 8% of the isolates using the Colispot test. This resistance was further confirmed by minimum inhibitory concentration (MIC) assays in liquid medium, which showed MIC values exceeding 2 μg/mL, confirming true colistin resistance according to EUCAST criteria.

Conjugation experiments demonstrated the plasmid-borne nature of amoxicillin resistance in all tested isolates. Nalidixic acid resistance was found to be transferable in two strains, while chromosomally encoded in another two.

Statistical analysis using the chi-square test revealed a significant correlation between antibiotic resistance and virulence traits among APEC strains.

Overall, these findings highlight the alarming rise in virulent and multidrug-resistant APEC strains within the poultry sector. Such strains represent a potential zoonotic threat and emphasize the urgent need for rational and regulated antibiotic use in poultry farming to mitigate risks to both animal and public health.

4.14. Haematological Dysregulation and Tissue Tropism in Adult Clarias gariepinus Experimentally Infected with Aeromonas hydrophila

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Introduction

As a key aquaculture species in Nigeria, *Clarias gariepinus* faces disease threats in its production. We studied the pathogenesis and pathology of an *Aeromonas hydrophila* isolate from a natural outbreak by experimentally infecting adult *Clarias gariepinus*.

Methods

Aeromonas hydrophila isolated from the dorsal kidney of a dead *Clarias gariepinus* during a disease outbreak was further propagated in tryptone soy broth. In total, 1 mL of an overnight broth culture of *A. hydrophila* (1.4×10^8 CFU/mL) was inoculated intraperitoneally into adult *Clarias gariepinus* (n = 50). A control group of 10 adult *Clarias gariepinus* was inoculated with 1 mL sterile saline intraperitoneally. Analyses included haematology, serum protein chemistry, postmortem, histopathology, immunohistochemistry, descriptive (Frequency tables) and inferential statistics (independent *t*-test).

Results

Aeromonas hydrophila induced a severe, fulminating disease and mortality in experimentally infected fish. Pathology revealed the liver, kidney, and gills to be the primary targets with infected fish exhibiting hepatic vacuolar degeneration, acute nephritis, hyperplasia and fusion of gill secondary lamellae, widespread haemorrhages, and ascites. Key haematological and serum chemistry findings included leukocytosis (WBC 14,766.06 ± 2560/μL vs. control 12,840.00 ± 199.69/μL), thrombocytopenia (PLT 145,151.56 ± 29,006.60/μL vs. control 234,900.00 ± 47,146.23/μL) and hypoalbuminemia (ALB 1.36 ± 0.35 g/dL vs. control 1.85 ± 0.81 g/dL) at 24 h post-infection in adults. The leukocytosis persisted at day 18; however, hyperglobulinemia was detected (GLB 5.07 ± 0.26 g/dL vs. control 4.80 ± 0.35 g/dL). Immunohistochemistry confirmed widespread tissue tropism with intense staining for *A. hydrophila* antigens in the skin and kidneys.

Conclusion

The haematological profile of *A. hydrophila* at 24 h is one of acute inflammatory response with marked thrombocytopenia, probably secondary to disseminated intravascular coagulation. The widespread haemorrhages observed could be linked to this pathophysiology. The key findings in the subacute to chronic phase suggest persistent inflammation or immune response. These results demonstrate a complex host–pathogen interaction where *A. hydrophila* employs cytotoxic virulence factors to cause significant tissue damage and haematological dysregulation.

4.15. In Vitro Investigation of Probiotics' Antimicrobial Effects on Bacteria That Cause Uterine Infections in Cattle

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Treating uterine infections in cattle is a challenge for farmers and veterinarians. Postpartum is the primary time for uterine infections, such as endometritis and metritis. They decrease fertility, have an impact on reproductive health, and lead to significant economic losses. The genital microbiota represents an opportunistic field of study in the area of cattle fertility. However, intravaginal antibiotic treatment affects the genital microbiome, leading to dysbiosis. Consequently, the modulation of this vaginal microbiome can be an effective alternative to antibiotics.

This study aims to to explore the potential use of bacteria as natural probiotics for the prevention of uterine infections and the improvement of reproductive health in dairy cows.

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Seven Montbeliarade dairy cows were involved in this study: one with endometritis, one with metritis, and five healthy heifers. Various culture mediums were used to seed vaginal swab samples. The antibacterial activity of lactic acid bacteria against the infectious agents was investigated using two methods. Eight antibiotics were tested against the infectious agents using the MH diffusion method, and the results were recorded in an antibiogram.

Using phenotypic identification, *Salmonella* (E1), *E. coli* (E2), and *Lactobacillus* (E3, E4) from the vagina of healthy cows and a mixture of commercial probiotic *L. plantarum* and *P. acidilactici* (E5) were obtained. E4 and E5 exhibited moderate antimicrobial activity against *E. coli*, but none against *Salmonella*. E5 (4 mm) was tested by the overlay method against *E. coli*. Isolate E4 also exhibited moderate activity (2 mm). *E. coli* was found to be very sensitive to several antibiotics, particularly to aztreonam (32 mm) and gentamicin (28 mm), while *Salmonella* showed partial resistance.

These results underline the importance of characterizing probiotic strains prior to their application. Lactic acid bacteria have the potential to be probiotics, and more in vitro and in vivo studies are necessary to confirm the stability, safety, and efficacy of the strains in clinical settings.

4.16. Influence of Urbanization on Gastrointestinal Parasites in Colonial Waterbirds

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Helminth parasites are key regulators of host health, population dynamics, and ecological interactions. Yet, the influence of urbanization on parasite prevalence in colonial waterbirds remains poorly understood. We investigated gastrointestinal parasites in *Ardeola grayii* across urban and semi-urban sites in North Kerala, India. A total of 150 fecal samples were collected and examined and we evaluated colony-level variables including site type (urban vs. semi-urban), nest abundance, and species composition (single vs. multi-species nesting).

Overall, the majority of samples (67.3%) were parasite-free, with a higher prevalence recorded in semi-urban sites (38.0%) compared to urban sites (24.1%). Logistic regression analysis revealed site category as a significant predictor ($p = 0.01$), indicating that birds in urban colonies had a lower probability of harboring parasites. In contrast, nest abundance ($p = 0.10$) and species composition ($p = 0.60$) showed no significant influence.

The lower prevalence of parasites in urban colonies likely reflects ecological shifts driven by urbanization. Contributing factors may include declines in intermediate host populations, altered foraging behavior of urban-adapted birds that rely on human-provided food, and potential pharmaceutical contamination in urban waterbodies that reduces parasite survival. Such findings align with broader ecological evidence that urbanization reduces biodiversity and host richness, thereby limiting opportunities for parasite transmission. This study underscores the importance of integrating parasite ecology into urban wildlife research. A deeper understanding of how urbanization shapes host–parasite interaction has significant implications for biodiversity conservation and for anticipating and managing zoonotic disease risks in rapidly developing landscapes.

4.17. Insights into the Intestinal Pathogenesis of Peste des Petits Ruminants in Sheep and Goats

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Background: Peste des petits ruminants (PPR) is a highly contagious and economically important viral disease of sheep and goats, posing a major threat to livestock production and food security in developing regions. While pulmonary and systemic manifestations of PPR virus (PPRV) infection are well documented, the intestinal pathology remains poorly described.

Methods: Twenty-one small ruminants (15 goats and 6 sheep) of varying ages were examined for clinical signs, gross lesions, and intestinal pathology. Intestinal samples were processed for histopathology, histomorphometry, and immunohistochemistry to detect PPRV antigen and inflammatory cytokines (IL-1 and NF-κB). Data were analyzed descriptively and inferentially at $\alpha = 0.05$.

Results: Most affected animals (85.7%) were $\leq$1 year old. Sheep exhibited more severe gastrointestinal signs (89%) than goats (63%), while goats showed greater systemic and respiratory involvement (91%). Intestinal lesions such as button ulcers and zebra stripes were more frequent in sheep (77%) than goats (59%). Histopathology revealed variable villous atrophy, stunting, matting, clubbing, forking, and necrosis, with sloughing of enterocytes and cryptal cell necrosis. Some cases showed cryptal hyperplasia and submucosal lymphocytic infiltration, suggestive of early infection. Notably, mucosal-associated lymphoid tissue hyperplasia and cytokine (IL-1, NF-κB) expression were more pronounced in goats. PPRV antigens were strongly immunolocalized in both enterocytes and cryptal cells, confirming viral replication in intestinal tissues.

Conclusion: PPRV infection produces significant enteric pathology in small ruminants. Goats exhibit stronger mucosal and cytokine responses than sheep, indicating species-specific differences in intestinal pathogenesis and immune modulation.

4.18. Intracellular Mechanism of Experimental Immunosuppression: A Comparative Study in T Cells and Macrophages

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Immunosuppression, characterized by a diminished ability of the immune system to combat infections, plays major role in modulating immune functions. Experimentally induced immunosuppression using pharmacological agents, is instrumental in unraveling key immune regulatory mechanisms and identifying potential therapeutic targets. In this study, we investigated the immunosuppressive effects of two drugs, Telmisartan (TM) and Tacrolimus (FK506) on metabolic markers of T cell and macrophage function, intracellular calcium, nitric oxide (NO), reactive oxygen species (ROS), and mitochondrial membrane potential (MMP). Telmisartan, an angiotensin II type 1 receptor blocker primarily prescribed for hypertension, has demonstrated emerging immunosuppressive properties. FK506, a calcineurin inhibitor, is widely used as a potent immunosuppressant. Despite advances in understanding the mechanisms of immunosuppression, metabolic alterations in immune cells under these conditions remain insufficiently characterized. Calcium signaling is essential for T cell activation, whereas NO is known to inhibit T cell proliferation, oxidative stress and mitochondrial integrity have emerged as critical indicators of immune status, as observed in various studies. We employed RAW 264.7 murine macrophage cell line and primary splenic T cells isolated ex vivo from 8 week old C57BL/6 mice, and subsequently analyzed in vitro. Our results found, intracellular calcium levels increased in activated splenic T cells and macrophages during immunosuppression. NO levels were reduced in experimentally activated T cells, but telmisartan increased NO

production, while tacrolimus showed a mild effect. In macrophages, activated cells showed increased NO levels, with telmisartan further enhancing NO production supporting its role in NO mediated antihypertensive effects. ROS levels were also increased during immunosuppression in macrophages. In T cells, MMP showed time dependent changes. Experimental activation induced MM polarisation, however drug treatment decreased polarization, indicating restoration of MMP via immunosuppression Our findings highlight that immunosuppressive drugs reprogram immune cell metabolism in a cell type specific manner rather than causing uniform immune suppression.

4.19. Metabolomic Insights into the Anti-Infective Potential of Azadirachta indica: An In Vivo and In Vitro Investigation

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Background: The escalating threat of multidrug-resistant (MDR) pathogens underscores the urgent need for novel anti-infective therapeutics. *Azadirachta indica* (Neem), a traditional medicinal plant, is rich in bioactive limonoids, quercetin derivatives, and azadirachtin with demonstrated antimicrobial activity. However, its metabolomic signature and translational anti-infective potential remain underexplored.

Methods: A combined in vitro and in vivo approach was employed. Methanolic Neem leaf extract was fractionated by LC-MS/MS–based untargeted metabolomics, identifying key metabolites linked to antimicrobial bioactivity. In vitro susceptibility was assessed against MDR *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using CLSI-standard broth microdilution assays. Synergy with ciprofloxacin and meropenem was evaluated through checkerboard assays. For in vivo validation, BALB/c mice (n = 48) were challenged intraperitoneally with MDR *E. coli*. Neem extract nanoparticles (50 mg/kg, oral gavage) were administered daily for 10 days, with ciprofloxacin (20 mg/kg) as positive control.

Results: Metabolomic profiling revealed abundant nimbolide, azadirachtin, and quercetin glycosides as major antimicrobial metabolites. Neem extract demonstrated potent in vitro activity with MICs of 64 μg/mL (*E. coli*), 32 μg/mL (*S. aureus*), and 128 μg/mL (*P. aeruginosa*). Checkerboard assays revealed synergistic interactions with ciprofloxacin (FICI = 0.28) and meropenem (FICI = 0.32). In vivo, Neem-treated mice exhibited a 65% survival rate compared to 25% in untreated controls (p 0.01). Bacterial load in spleen and liver decreased by >1.8 log CFU, with concomitant reduction in serum TNF-α (↓42%) and IL-6 (↓37%).

Conclusion: Neem demonstrates potent anti-infective efficacy through synergistic interactions with antibiotics, immune modulation, and direct antimicrobial activity. Metabolomics-guided identification of active compounds positions *Azadirachta indica* as a promising medicinal lead for combating MDR infections.

4.20. Molecular Characteristics of Biofilm Forming Gene (algL) in Nosocomial Pseudomonas aeruginosa and Their Inhibition by Tomato Carbon Dots and Manuka Honey

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Microbial biofilm buildup poses a significant challenge in hospital intensive care units (ICUs), by clogging healthcare devices, consequently resulting in treatment failure.

The current work aimed to isolate biofilm-forming *Pseudomonas aeruginosa*, sequence and analyze the biofilm-forming gene (algL), and evaluate the biofilm-inhibitory potential of tomato carbon dots and manuka honey. For biofilm formation and inhibition analysis, the microtiter plate, Congo red, and tube methods were used. A total of 150 samples (swabs) from catheter mounts, ventilator masks, and urine catheters were obtained and processed in the ICUs of three hospitals in the district of Peshawar, Pakistan. *P. aeruginosa* was identified in 45 samples. The antibiogram of *P. aeruginosa* isolates demonstrated resistance to tobramycin (53.4%), ciprofloxacin (68.8%), aztreonam (42.2%), co-trimoxazole (60.6%), cefepime (40%), and ceftazidime (40%). Biofilm formation was detected in 95.5% of the samples. The biofilm was inhibited by up to 86.40% with 100 mg/mL of tomato carbon dots, whereas 68.95% inhibition was observed at 100 mg/mL of manuka honey. A total of 73.1% of *P. aeruginosa* isolates were positive for the presence of the algL gene. Mutational analysis revealed that the algL gene was conserved across all isolates. However, each isolated testis harbored one of the previously identified missense mutations in the algL gene. It is important to investigate the crucial factors involved in biofilm formation to develop strategies for preventing clogging of medical devices used for therapeutic purposes. Current findings may help pave the way for the development of new anti-biofilm methodologies and, in turn, effective antimicrobial therapies. Mutational analysis could yield significant insights into biofilms and inform effective inhibition strategies.

4.21. Morpho-Functional Insights into Sparicotyle chrysophrii: The Mediterranean Aquaculture Scourge

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The polyopisthocotylean ectoparasite *Sparicotyle chrysophrii* is causing devastating effects on gilthead seabream net-pen farms across the Mediterranean. Adult parasites attach to host gills by the haptor, rigged with dozens of specialized clamps, and feed on fish blood. Severe anemia occurs during high-intensity outbreaks, which provoke direct mortalities, secondary infections and lower fish performance. Fundamental aspects of the parasite's physiology and morphology remain poorly understood.

Livee adult parasite specimens were retrieved from the gills of experimentally infected gilthead seabream. Parasites were fixed for histology, scanning and transmission electron microscopy (SEM, TEM), as well as confocal microscopy (CM). Histological worm sections, ultrathin sections (TEM), whole specimens (SEM), and fluorescent-labelled actin and tubulin filaments in whole mounts (CM) were examined.

Sensory, reproductive, and secretory/excretory structures were identified. The cephalic region gathers the highest concentration of presumably sensory cilia involved in feeding, whereas microvilli in the remaining parasite body might serve for host encounter and/or orientation with regard to water flow. The parasite's strong ability to lengthen/stretch and shorten/round their body is the result of the contraction of the longitudinal muscle fibers, which most probably contribute to the parasite's exploratory behavior and/or interaction with other specimens. Further strong muscle fiber arrangements were also observed in the oral apparatus, male reproductive organ, and clamps. Additionally, specialized digestive cells involved in hemoglobin processing were identified.

Many of the observed morphological features have paramount roles in the parasite's essential biological functions, such as feeding, host encounter, and spreading success. Deeper understanding will contribute to shedding some light on the host–parasite interaction and in turn aid in the development of effective and targeted control strategies against sparicotylosis.

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4.22. Multidrug Resistance and ESBL Gene Profiles of Enterobacteriaceae in Hospital Wastewater from Dhaka, Bangladesh

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Introduction: Hospital wastewater is a major environmental reservoir for antibiotic-resistant bacteria (ARB) and resistance genes. In rapidly urbanizing regions like Dhaka, inadequate wastewater treatment enables the uncontrolled release of multidrug-resistant (MDR) pathogens into natural water bodies. Enterobacteriaceae, including *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella* spp., serve as key indicators of antimicrobial resistance (AMR) in healthcare effluents. Discharge of such effluents facilitates the environmental spread of Extended-Spectrum Beta-Lactamase (ESBL)-producing strains. This study aimed to isolate, identify, and characterize Enterobacteriaceae from hospital wastewater, focusing on their phenotypic resistance patterns and molecular mechanisms underlying β-lactam antibiotic resistance.

Methodology: Wastewater samples were collected from major hospitals in Dhaka. Forty Enterobacteriaceae isolates were recovered using selective media and identified via biochemical tests (IMViC, urease, H_2S, motility, lactose fermentation) and the API 20E system (>88% accuracy). Antibiotic susceptibility against 15 clinically relevant drugs, including β-lactams, carbapenems, and aminoglycosides, was assessed using the Kirby–Bauer disc diffusion method. Representative ESBL-producing isolates underwent PCR to detect bla-TEM, bla-SHV, and bla-CTX-M genes, allowing correlation of genotypic and phenotypic resistance.

Results: Predominant isolates included *E. coli, K. pneumoniae, Salmonella Typhi, Shigella* spp., *Proteus* spp., and *Enterobacter* spp. High resistance was observed against Amoxicillin (44.44%), Ceftazidime (41.67%), and Ceftriaxone (38.89%), with lower resistance for Meropenem (19.44%) and Gentamicin (16.67%). ESBL production was detected in 53.5% of isolates, 44.34% were MDR, and 11.20% XDR. PCR revealed bla-TEM in 75% of ESBL-positive isolates, while bla-SHV and bla-CTX-M were each found in 25%, indicating active horizontal gene transfer.

Conclusion: Dhaka hospital wastewater serves as a significant reservoir of MDR and ESBL-producing Enterobacteriaceae. The predominance of bla-TEM emphasizes its role in environmental dissemination of β-lactam resistance. These findings highlight the urgent need for systematic AMR surveillance, antibiotic stewardship, and advanced wastewater treatment to mitigate environmental and public health risks.

4.23. Multiparametric Characterization of Gastrointestinal-Based Parasites and Associated Pathology Lesions in Ruminants Slaughtered at Akinyele Abattoir, Oyo State, Nigeria

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This study investigated gastrointestinal parasites and ticks in cattle and goats at Akinyele Abattoir, South-Western Nigeria, to address gaps in epidemiology, species identification, and pathology. Faecal samples and adult parasites were collected from 61 cattle

and 35 goats, between March and July 2025. The employed morphological techniques and 18S rRNA gene sequencing confirmed the presence of Strongyles, *Eimeria* spp., *Fasciola gigantica*, *Paramphistomum cervi*, and *Moniezia expansa*. Strongyles dominated across hosts, with higher burdens in Red Bororo and White Fulani cattle, and Red Sokoto and West African Dwarf goats. The multinomial logit model revealed the susceptibility of males and younger animals to multiple infections. Goats carried higher egg/oocyst counts than cattle. Gross lesions in cattle included mucosal hyperaemia, catarrhal to haemorrhagic enteritis, and nematode presence, while goats exhibited mucosal reddening, thickening, and catarrhal enteritis, with severe pathology linked to dictyocaulosis and schistosomiasis, caused by *Dictyocaulus* spp. and *Schistosoma* spp., respectively, or chronic fluke infection. Molecular analysis confirmed *Fasciola gigantica*, *Paramphistomum cervi*, and *Moniezia expansa* with 99–100% sequence identity, supporting phylogenetic relationships. *Fasciola* was absent in cattle but found in goats. Our findings highlight breed, sex, and age vulnerabilities; the value of combined diagnostics; and the need for targeted deworming, pasture hygiene, and selective breeding, to improve livestock health and reduce zoonotic risks. Future research addressing seasonal dynamics, anthelmintic resistance, genetic markers of host resistance, and spatial determinants of parasite exposure to strengthen livestock health and One Health outcomes is encouraged.

4.24. Newcastle Disease Across Different Taxa of Parrots (Psittaciformes): Current Knowledge, Pathogenesis, and Preventative Strategies

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Newcastle Disease (ND), caused by Avian Paramyxovirus type 1 (APMV-1), remains a major viral threat to global poultry production. While ND has been extensively studied in domestic chickens, knowledge regarding its occurrence and impact in parrots (Psittaciformes) is still limited. The literature records demonstrate that ND can infect at least 22 parrot species across two major superfamilies, Psittacoidea and Cacatuoidea, with the potential involvement of lentogenic strains in Strigopoidea. In Indonesia, reports are scarce, but several cases documented since the early 20th century confirm infections in cockatoos (*Cacatua* spp.). Clinical manifestations in Psittaciformes vary and include respiratory distress, neurological symptoms such as torticollis and paralysis, and histopathological lesions of the myocardium, including lipomatosis, hemorrhage, bacterial colonization, and loss of myofibrillar striation. Epidemiological risk factors are associated with the role of feral birds as reservoirs, the illegal and legal bird trade, and the potential for interspecies mutations that facilitate viral spillover. Effective treatment strategies for ND in parrots remain unavailable, making prevention the cornerstone of disease management. Preventive measures include strict biosecurity protocols, sanitation with lipid solvents and exposure to heat or solar radiation, and vaccination with lentogenic or mesogenic strains. However, vaccination programs are not uniformly applicable across all Psittaciformes species due to physiological and management differences. Overall, this review emphasizes that despite limited case reports, parrots are significantly susceptible to ND, and further research is required to clarify prevalence and pathogenesis and to develop tailored preventive strategies for this diverse avian group.

4.25. Novel, Sensitive, and Specific Diagnosis of Hookworm from Urine Samples

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Hookworm is one of the most prevalent neglected tropical diseases in the world, infecting nearly 1.5 billion people worldwide. Severe cases of hookworm can lead to severe anemia, which can cause developmental complications in young children. The current method of hookworm diagnosis involves egg detection from stool samples via a Kato–Katz smear under a microscope, which is neither efficient nor sensitive. Hookworm diagnosis involving amplification of species-specific cell-free DNA from urine samples is feasible, as hookworm DNA can be filtered out of urine. The goal is to demonstrate this feasibility so that working with stools can be avoided in the future. Different concentrations of ITS-2 and repeat primers for the amplification were tested, and 250 nM consistently demonstrated the best amplification. Six urine samples of approximately 40–50 mL were collected from Marquette University volunteers. Nine different serial dilutions of genomic DNA (gDNA) of Necator americanus and Ancylostoma duodenale, demonstrating high, medium, and low concentrations, were prepared (3 different concentrations × 3 serial dilutions of each concentration = a total of 9 combinations). The nine combinations for each species were then filtered using Whatman grade 3 filter paper, air dried, and packed in a Ziploc bag with desiccant. Then, DNA was extracted using the QIAMP Blood Mini Kit and Nanodrop for concentration. These extracted DNAs are now being amplified using species-specific primers for both the ITS-2 gene and repeat fragments, including negative and positive controls, and will be visualized using gel electrophoresis. This is a novel assay that will reduce the need for using stools in the future, leading to a faster, more sensitive, and more specific assay that can help facilitate targeted mass drug administration.

4.26. Pathology and Aetiology of Pneumonia in Nigerian Sheep: Evidence of Viral–Bacterial Co-Infections and Breed Susceptibility

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Background: Current vaccination and therapeutic strategies have not effectively controlled pneumonic cases in sheep. A better understanding of the nature, pathology, and etiological factors of ovine pneumonia is essential for improved prevention and management, particularly in Nigeria.

Methods: A total of 828 sheep from different regions of Nigeria were evaluated ante- and post-mortem for pulmonary consolidation in relation to breed, age, sex, clinical signs, and body condition. Lung samples were examined using histopathology, bacterial culture, and bronchoalveolar lavage fluid (BALf) cytopathology. Paraffin-embedded tissues were analyzed immunohistochemically for viral antigens—Peste des petits ruminants virus (PPRV), Parainfluenza-3 virus (PI3V), Respiratory syncytial virus (RSV)—and bacterial antigens—Mannheimia haemolytica (Mh), Pasteurella multocida (Pm), and Mycoplasma mycoides var. capri (Mmvc). Data were analyzed using descriptive statistics and ANOVA at $\alpha = 0.05$.

Results: Pneumonia occurred in 56% of sheep, with higher susceptibility in Yankasa breed (38%) and females (39.5%). Histopathological patterns included interstitial (24%), bronchointerstitial (18%), bronchopneumonia (12%), and granulomatous pneumonia (2%). BALf showed elevated neutrophils, lymphocytes, and macrophages in pneumonic lungs. The predominant bacterial isolates were *E. coli*, Mh, and Pm. Immunohistochemistry revealed strong viral antigen staining in bronchointerstitial pneumonia and bacterial antigen in bronchopneumonia. Co-infections were frequent, particularly PPRV with Mh (63%).

Conclusion: Pneumonia is common among young and Yankasa sheep in Nigeria, with PPRV and RSV as key viral agents, often complicated by *M. haemolytica*. Effective control may require a multivalent vaccine incorporating these pathogens.

4.27. Predictive Hematological Modeling for Field-Based Diagnosis of Haemoparasitic Infections in Ruminants in Resource Limited Setting

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Objective: This study leveraged statistical modeling and clinical pathology to develop a predictive framework for field diagnosis due to significant impairment of livestock productivity in tropical regions with Haemoparasitic infections but their diagnosis in resource-limited settings remains challenging.

Materials and Method: A cross-sectional analysis of 112 ruminants (57 cattle, 55 small ruminants) at an abattoir in Ibadan, Nigeria was conducted. Blood samples were collected for Giemsa-stained microscopy (definitive diagnosis) and full hematological profiling, including packed cell volume (PCV), hemoglobin (Hb), white blood cell count (WBC), platelet count, and neutrophil-lymphocyte ratio (NLR). Data were analyzed in R (v4.0+) using both parametric and non-parametric tests. Univariable and multivariable logistic regression were employed to identify hematological predictors of infection, with model performance quantified by Receiver Operating Characteristic (ROC) curves and Area Under the Curve (AUC) analysis.

Results: The analysis revealed that infection with *Trypanosoma*, *Babesia*, *Theileria*, or *Anaplasma* species caused significant hematological alterations: profound anemia (mean PCV drop: 21.4% vs. 30.8% in healthy, $p < 0.0001$), leukocytosis, elevated NLR (5.8 vs. 2.1, $p < 0.0001$), and thrombocytopenia (78 vs. 125 $\times$ $10^3/\mu L$, $p = 0.001$). Analytics derived a highly predictive diagnostic triad: PCV 24%, NLR > 4, and platelets 150 $\times$ $10^3/\mu L$. This composite metric achieved a >90% probability of detecting subclinical infection in critical cases (PCV 20%, NLR > 6, platelets 60 $\times$ $10^3/\mu L$), as validated by bootstrap resampling. Breed-specific baselines (e.g., resilient Ndama cattle vs. susceptible Red Bororo) were critical for accurate interpretation.

Conclusion: This study provides a robust, statistically-derived hematological model that serves as a cost-effective, field-deployable tool for the early triage and management of haemoparasitism, enabling data-driven decisions in settings without access to advanced diagnostics.

Keywords: Breed-Specific Susceptibility, Field Diagnostics Hemoparasitic Infections, Predictive Hematology, Ruminant Healt

4.28. Structure-Based In Silico Identification of Antimalarial Lead Compounds from Dioclea Reflexa Targeting Essential Plasmodium Falciparum Enzymes

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Malaria remains a global health crisis, primarily driven by drug-resistant Plasmodium falciparum strains, necessitating the urgent discovery of novel and safe antimalarial agents. The aim of this study was to identify potent inhibitors against four essential *P.*

falciparum enzymes: PfCullin-2, PfNADP-IDH, PfPKG, and PfAMA1. This study employed a structure-based in silico approach, combining molecular docking, drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analyses, to screen phytochemicals from *Dioclea reflexa*. Molecular docking results identified three potent lead compounds with high binding affinities (ΔG) often surpassing or equaling standard antimalarial drugs. The most potent hit was the phytosterol β-Sitosterol which showed a maximum affinity of −10.7 kcal/mol against PfCullin-2, exceeding the affinity of the control drug Atovaquone (−10.4 kcal/mol). Moreover, Lupeol (triterpenoid) was a dual inhibitor, demonstrating significantly superior binding to PfNADP-IDH (−9.6 kcal/mol) and PfAMA1 (−8.4 kcal/mol) compared to Primaquine (−5.7 and −6.6 kcal/mol, respectively). The flavonoid 7,4′-Dihydroxyflavone exhibited a docking score of −8.9 kcal/mol against PfPKG, comparable to the reference drug Artemisinin (−9.2 kcal/mol). Pharmacokinetic evaluation revealed a trade-off between potency and drug-likeness. While β-Sitosterol and Lupeol are the most potent binders against the malaria target receptors, their high lipophilicity resulted in one Lipinski violation and predicted poor aqueous solubility. In contrast, 7,4′-Dihydroxyflavone showed zero Lipinski violations and good solubility, establishing it as the most drug-like candidate. Toxicity screening predicted all three compounds to be inactive for major risks including hepatotoxicity, mutagenicity, and carcinogenicity. However, β-Sitosterol and Lupeol were flagged for neurotoxicity, while 7,4′-Dihydroxyflavone was flagged for nephrotoxicity.

4.29. The Effect of Selenium Nanoparticles and Cross-Talk of Selenium Nanoparticles and Fusarium oxysporum f. sp. lupini on the Concentration of Phytohormones in Germinating Seeds and Seedlings of Lupinus luteus L.

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[Aims]: The application of selenium nanoparticles (SeNPs), including mitigating biotic stress factors in plants, was discussed, but the influence of SeNPs on yellow lupine growth and the concentration of defence-related phytohormones such as jasmonic acid (JA) and salicylic acid (SA), during the seed germination stage and development of the seedlings of *Lupinus luteus* L. cv. Diament and *Lupinus luteus* L. cv. Mister, and during the cross-talk of SeNPs and pathogenic fungus *Fusarium oxysporum* f. sp. *lupini*, has not been studied. Additionally, the aim of this study was also to investigate the changes in concentrations of phytohormones such as giberelic acid (GA3 and GA4) and indole 3-acetic acid (IAA), playing important roles in the seed germination stage. Moreover, it was important to check

whether the application of SeNPs had an impact on limiting the development of infection and Fusarium wilt. [Methods]: The experimental protocol used in this study was based on a model system: germinating seeds pretreated with SeNPs, or not pretreated, were inoculated with an *F. oxysporum* f. sp. *lupini* spore suspension or non-inoculated, and were transferred to hydroponic grow boxes containing Hoagland medium. [Results]: The analytical results of the QuEChERS method showed significant changes in concentration of defence-related phytohormones and GA3, GA4 and IAA concentrations, in SeNPs, Fusarium and SeNPs + Fusarium variants, in both Diament as well as Mister varieties. Moreover, the results of our research demonstrated that SeNPs induced better turgor of the embryo axes and the roots and limited the development of infections and fusariosis in comparison to the control. [Conclusions]: The results provide a basis for further understanding the mechanisms of interaction of SeNP itself as well as SeNPs and *F. oxysporum* in the host plant.

5. Zoology

5.1. Oxidative Stress and MTF-1/Nrf2/JNK/Metallothionein B Network Restricts Pb Load in Drosophila Melanogaster

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Lead (Pb) is among the most severe and hazardous heavy metal pollutants. However, the complex response and detoxification mechanisms employed by insects to counteract Pb exposure remain incompletely elucidated. Using *Drosophila melanogaster* as a model, we integrated Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), transcriptomic analysis, Drosophila genetic tools, and a series of physiological assays to systematically investigate the signaling networks underlying the insect Pb detoxification response. Our findings revealed that Pb accumulation in *D. melanogaster* followed a nonlinear pattern with increasing dietary Pb concentration. Further mechanistic exploration demonstrated that Pb-induced high expression of Metallothioneins (Mtns), especially the MtnB, directly accelerated Pb excretion, thereby restricting the body's Pb burden and ultimately contributing to the nonlinear accumulation pattern. To validate this role, we employed an optogenetically controlled bacterial system to drive MtnB expression specifically in the *Drosophila* midgut; this manipulation effectively suppressed Pb accumulation in the flies and mitigated Pb-induced toxicity. At the molecular level, Pb exposure significantly elevated ROS levels in the Drosophila midgut. This ROS burst subsequently induced Mtns expression through the concerted action of the MTF-1, Nrf2, and JNK signaling pathways. Notably, we observed that Pb exposure activated the Nrf2 pathway via oxidative stress, thereby conferring cross-resistance to pesticides in fruit flies. Collectively, these insights provide a novel perspective on the crosstalk between oxidative stress, metal metabolism, and detoxification systems in insects. They not only advance our understanding of how insects cope with metal toxicity but also highlight the broader environmental implications of metal pollution in ecological systems.

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5.2. A Comparative Microscopic Study on the Ultrastructural Patterns of Shed Snake Skin for Distinguishing Venomous and Non-Venomous Snake Species

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Snakes represent a taxonomically diverse group that plays a critical role in ecosystems and is associated with public health issues; however, accurate identification remains challenging in many resource-limited settings. We evaluated microscopic ultrastructures in the microdermatoglyphics of scales collected from shed-off skin as a non-invasive and cost-effective diagnostic method. Shed-off skins from twelve adult specimens—three each from two venomous cobras (*Naja naja*, *Naja kaouthia*) and two non-venomous colubrids (*Ptyas mucosa*, *Fowlea piscator*)—were collected from different geolocations in Bangladesh. Then, 15 types of scales representing head, body, and tail regions of snakes were collected and prepared for microscopic observation, and the micro-ornamentation of microdermatoglyphics was examined at 40X, 100X, and 400X magnifications. Six ultrastructural characters (edge perimeter, edge area, edge WPD, follicle perimeter, follicle area, follicle 20,000 μm^2) in microdermatoglyphics were quantified. Paired *t*-tests and Wilcoxon signed-rank tests ($p = 0.001$) assessed stability within species and variation between species, as well as group. The results show that 75.56%, 58.89%, 76.67%, and 77.78% of characters are stable across the 15 scales in *N. naja*, *N. kouthia*, *P. mucosa*, and *F. piscator*, respectively. Four characters (out of six) significantly ($p = 0.001$) differentiate *N. naja* from *N. kaouthia*, whereas all six characters separate *P. mucosa* from *F. piscator*. Furthermore, five characters significantly differentiate more than 50% of the analyzed scales of the venomous from the non-venomous groups. PCA accounted for 77.6% (PCA1 and PCA2) of the variance, with clear species-wise and group-wise clustering. This approach enables reliable differentiation between closely related congeneric species and far distant species, providing a practical alternative to conventional taxonomic techniques. It holds potential for application in field-based diagnostics, snakebite management, forensic herpetology, and biodiversity monitoring.

5.3. Breeding Biology of the Long-Tailed Shrike (Lanius schach) and Its Response to Brood Parasitism by the Asian Koel (Eudynamys scolopaceus)

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The Long-tailed Shrike (*Lanius schach*) serves as a key host for the brood parasitic Asian Koel (*Eudynamys scolopaceus*). This study aims to investigate the breeding success, nesting patterns, and response to brood parasitism. Surveys of Long-tailed Shrike nests were carried out from March to August 2024, covering the breeding season. We searched for nests or adult shrikes through both walking and bicycle surveys in regions that were dominated by shrike-preferred habitats. Breeding activity peaked in May (37.5%), while the lowest activity was recorded in March, June, and July (12.5%). No breeding activity was observed in August. A total of eight Long-tailed Shrike (LTS) nests were identified, of which six were active and contained at least one egg. In total, 50% of nests were parasitized, and 50% were unparasitized. Breeding success of the LTS in parasitized and unparasitized nests was recorded as 55.56% and 58.75%, respectively, in relation to hatchlings, and also 36.11% and 47.78%, respectively, in relation to fledglings. The highest percentage of nesting

materials commonly used by LTS for nest construction was fine grass (34.37%), followed by plastic threads (9.75%), ropes (7.45%), twigs (10.91%), leaves (0.51%), polythene (1.28%), root fibers (3.01%), synthetic nets (1%), cotton (0.22%), plant bark (0.73%), fabrics (1.20%), plastic bags (7.56%), and unidentified material (22%). The majority of nests (75%) were located at the periphery, and a few nests were built in central positions (25%) of the trees. Most nests were found in Mahogany trees, and the LTS primarily used Banana leaves (63.01%) for nest construction, but also used Acacia, Rain tree, and Mahogony leaves. These outcomes help to determine how brood parasitism affects host reproductive success. This study also highlights the urgent need for management of plastic pollution to reduce its integration into bird nests and to protect avian health.

5.4. Comparative Morphology of the Maxillary Dentition of 15 Neotropical Opisthoglyphous Snake Species Found in Paraná, Brazil

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The dentition of snakes serves a key function in their nutrition, as their feeding strategy is directly related to the shape and size of teeth in various dental bones. The morphology of snake's jaws is highly variable and can be considered an important source of taxonomic information. As such, dentition has been used for over a century to describe and identify taxa, continuing to be an important tool in both taxonomy and phylogeny. The goal of this study was to analyze the maxillary dentition of 15 species of neotropical opisthoglyphous snakes (*Clelia plumbea, Helicops carinicaudus, Oxyrhopus clathratus, Oxyrhopus guibei, Oxyrhopus rhombifer, Philodryas aestiva, Philodryas nattereri, Philodryas olfersii, Philodryas patagoniensis, Pseudoboa haasi, Siphlophis compressus, Thamnodynastes nattereri, Thamnodynastes strigatus, Tomodon dorsatus,* and *Tropidodryas striaticeps*), with an emphasis on species found in the state of Paraná, Brazil, as a system to assess intra- and interspecific differences among them. This study aimed to examine the maxillary morphology of snakes with different diets but all belonging to the same colubrid lineage. Additionally, it sought to evaluate the applicability of alternative techniques for osteological material preparation and to interpret the observed variation from functional (diet) and evolutionary perspectives, based on available phylogenies. Two preparation techniques were used: dry preparation and diaphanization methods. The analysis of the specimens was conducted using a Leica stereoscopic microscope, and data obtained were analyzed using PAST software. The results suggested that measuring the jaws alone was insufficient to determine the phylogeny or specialized diet of the assessed opisthoglyphous species.

5.5. Determinants of Butterfly Wing Eyespot Patterns: Possible Roles of Epithelial Cells and Cuticle Interactions

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Introduction

The extracellular matrix (ECM) plays a crucial role in cell differentiation by providing ligands that interact with receptors on the cell surface. The cuticle functions as a specialized form of ECM. In butterflies, wing patterns are formed by the precise arrays of scales. During the early pupal stage, the cuticle layer forms in close apposition to the epithelial cells that

will later differentiate into scale cells. In the blue pansy butterfly (*Junonia orithya*), the prospective eyespot pattern is pre-determined early in the pupal stage. The center of the future eyespot is recognized as a developmental organizer. Our laboratory discovered that the physical contact of the pupal wing epithelium with the cuticle plays an essential role in eyespot formation. Based on these observations, we hypothesized that the physical interactions between the epithelial cells and the cuticle contributes to the spatial regulation of eyespot pattern development.

Methods

We utilized three types of microscopes: a standard light microscope, a transmission electron microscope (TEM), and a confocal laser scanning microscope (CLSM). We mainly examined the width of the intervening spaces between the epithelial cells and the cuticle as an indicator of the physical interactions between them.

Results

We found that the width of the intervening space, cuticle thickness, and cuticle surface structures differed between the pupae of 6 and 12 h post-pupation in the forewings, based on the light microscopy and TEM observations. Furthermore, using a CLSM, we found that the center of the prospective hindwing eyespot is different from its surrounding regions in the width of intervening space in the pupae of 11–13 h post-pupation.

Conclusion

The width of the intervening space between the epithelial cells and the cuticle on the prospective eyespot center may be an indication of the behavior of the eyespot organizer for the determination of eyespot patterns.

5.6. Effect of Some Physicochemical Parameters on the Zooplankton Community of Lake Oubeira—A Ramsar Wetland in the Extreme East of Algeria

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This study aims to evaluate the variability of physico-chemical parameters and their influence on zooplankton communities in Lake Oubeira. The issue that arises in this context is how does the seasonal variability of physico-chemical parameters structure the spatiotemporal distribution of zooplankton communities in Lake Oubeira?

Two sampling stations were selected: station 1: Demnet Errihane north of the lake; station 2: Oued Messida south of Lake Oubeira. The physico-chemical parameters of the environment are measured in situ using a multiparameter, monthly from January 2021 to January 2022. The zooplankton sampling was carried out using a zooplankton net (mesh size = 50 μm), 50 L of lake water were filtered per sample to obtain a concentrated volume of 50 to 120 mL, 3 mL of the concentrate were subsequently observed under an optical microscope.

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After taxonomic identification and the enumeration of zooplankton individuals, statistical data analysis -software R- revealed that: the temperature reaches its maximum in summer and decreases in winter, while the dissolved oxygen concentration is higher in spring. As for the autumn, it is distinguished by high values of electrical conductivity and pH.

PERMANOVA test revealed a very highly significant effect of seasons on species distribution (p-value 0.001), while "stations" factor showed no significant effect.

The results of NMDS (Non-Metric Multidimensional Scaling) analysis indicated a marked seasonal structuring of communities. Copepods are predominantly abundant during the warm seasons, while rotifers and cladocerans predominate in colder seasons. Moreover, some species have a broader distribution, being observed over several seasons. At the end of this research work, we can confirm that physico-chemical parameters such as temperature, pH, dissolved oxygen, electrical conductivity strongly structure the zooplankton communities of Lake Oubeira. These results highlight the role of zooplankton as a valuable bioindicator of ecosystem health through its sensitivity to environmental change.

5.7. Evolution of Prey Transport in Marine Birds: Integration of Ethology and Funtional Morphology

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Food transport represents a key behavioral process enabling the ingestion of nutrients and water. The behaviors and mechanisms underlying food acquisition are closely linked to sensorimotor actions that emerge from the integration of both morphological heritage and environmental constraints. In accordance with the actual framework proposed in the literature, food transport can be interpreted as a complex sequence of coordinated movements involving cranial and postcranial structures, whose evolution reflects functional trade-offs between efficiency, ecological opportunity, and phylogenetic constraint. Following the broader eco-evolutionary perspective, food transport behaviors should also be understood in the context of environmental variability and resource accessibility, which shape the selective pressures driving behavioral flexibility and innovation. This paper provides an overview of current knowledge on the evolution of behavioral and functional patterns associated with food transport in birds exploiting marine food resources. We compare different modes of food transport across major avian lineages—particularly shorebirds (Charadriiformes), Anatidae, and Laridae—in order to highlight the diversity of feeding strategies and their functional bases in relation to ecological specialization. By integrating behavioral and functional approaches within a phylogenetic framework, we aim to reconstruct the evolutionary scenarios underlying food transport in marine birds. This synthesis allows us to test hypotheses concerning ancestral behavioral patterns, convergence, and the adaptive landscape that has shaped the evolution of food transport mechanisms in marine avifauna.

5.8. Invasion and Expansion of Monogenean Parasites in India: Emerging Threats to Fish Biodiversity and Health

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The invasion of non-native parasitic fauna in India has steadily increased alongside the introduction of their exotic hosts. The expansion of non-native monogeneans in Indian waters has been largely facilitated by human-mediated transfers of these hosts, which play

a major role in their establishment in new localities. Parasites of exotic hosts may switch to native species, leading to transmission that can adversely affect India's native fish diversity.

However, limited attention has been given by Indian researchers to non-native monogenean parasites, as no such species had been documented previously. In recent years, growing interest in biological invasions has encouraged biologists to investigate the role of non-native parasites and the risks associated with them. Based on recent occurrences of monogeneans in India, it is evident that these parasites are co-introduced with their exotic hosts and readily establish themselves in new environments due to their simple, single-host life cycle.

The aquarium fish trade is a major pathway enabling the continual introduction and spread of monogenean parasites in India. The presence of non-native monogenean fauna indicates a lack of effective national guidelines for the ornamental fish trade. Therefore, proper surveillance and disease diagnostic measures should be implemented prior to the introduction of exotic fishes. Additionally, existing Indian guidelines for the import of ornamental species should be revised to safeguard both aquaculture industries and the aquatic environment.

The objective of this study is to assess the extent of non-native monogenean parasite invasions in Indian waters and to analyze the associated risks to native fish species.

5.9. Linking Estrogen Receptor Expression to Pubertal Onset in Duolang Sheep Across Levels of Biological Organization

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Puberty in sheep represents the activation of the hypothalamus–pituitary–ovary axis, but how estradiol (E2) and its receptors coordinate this transition is still unclear, especially in early-maturing indigenous breeds. Duolang sheep from arid Xinjiang reach first estrus unusually early, offering a natural model to link hormone dynamics, receptor expression, and breed-specific reproductive adaptation across biological levels. Eighteen Duolang ewe lambs were allocated to three stages (prepuberty, puberty, and postpuberty; $n = 6$ per group). In addition to measuring ERα and ERβ mRNA and protein in the hypothalamus, pituitary, and ovary, we also measured serum E2. Primary ovarian granulosa cells were cultured and exposed to graded E2 concentrations (0–1000 ng/mL) to assess dose-dependent changes in ERα, ERβ, and gonadotropin-releasing hormone (GnRH) expression. Around first estrus, circulating E2 displayed a sharp peak. E2 spiked around first estrus and fell afterward. ERα expression in the hypothalamus and pituitary decreased during puberty and increased post-puberty, indicating a shifting negative feedback role of E2 on the central axis. Conversely, ovarian ERα peaked during puberty and increased gradually from pre- to postpuberty. ERα expression in granulosa cells was biphasic, peaking at 250 ng/mL E2 concentration, while ERβ and GnRH levels increased with higher E2 doses. ERα is the main mediator of E2 feedback in the hypothalamus-pituitary axis, while ERβ is more involved in ovarian maturation and GnRH regulation during pubertal transition. Our study connects molecular receptor dynamics, tissue-level signaling, and breed-

level reproductive phenotype, providing candidate biomarkers for selecting high-fertility, early-maturing sheep.

5.10. Microdermetoglyphic Morphometry of Shed-Off Skin: A Novel Approach to Solving Snake Identification Complexity

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Accurate snake identification is fundamental for ecological research, biodiversity conservation, and snakebite management, particularly when dealing with medically important venomous species. Traditional morphological methods, however, are often unreliable and pose serious envenomation risks during live handling, while molecular approaches—though precise—require costly infrastructure and technical expertise, limiting their accessibility in many regions. These challenges highlight the urgent need for a safer, reliable, and field-friendly alternative. In this study, we present a **novel, non-invasive identification method** based on the **micro-dermatoglyphic features of shed snake skin**. Shed skins, commonly deposited in safe habitats during ecdysis, are readily detectable in the field and can retain microscopic structural traits for extended periods. We quantified six micro-dermatoglyphic features *(edge perimeter, edge area, edge widest part distance, follicle perimeter, follicle area,* and *number of follicles per 20,000 μm^2*) from three head, nine trunk, and three tail scales in seven individuals each of *Naja kaouthia* and *Naja naja*. Statistical analyses demonstrated that these features not only reliably distinguish between the two species but also differentiate between sexes. Among the traits, *edge widest part distance* emerged as the most powerful discriminator, correctly separating 93.3% of scales between species (p 0.001), followed by *edge area* (86.7%) and *number of follicles per 20,000 μm^2* (46.7%). Furthermore, sex-based differences were significant (p 0.001), with *edge area* distinguishing 60% of scales in *N. kaouthia* and 20% in *N. naja*. This study introduces the first demonstration of quantitative, rather than qualitative, **shed-skin microdermatoglyphics as a taxonomic and ecological tool**. By eliminating the risks of live handling and bypassing molecular constraints, this approach offers a **practical, non-invasive, and widely applicable method** for snake identification. Beyond taxonomy, it holds promise for evolutionary studies, biodiversity monitoring, and informing conservation and public health strategies in snakebite-prone regions.

5.11. Morphology of the Skin Scales and Sense Organs of Anolis Carolinensis

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Although the skin-scale microstructures of squamate reptiles are known to be highly adapted for habitat-specific functions, including protection, camouflage, and thermoregulation, the structural adaptations of the skin of the color-changing ectotherm *Anolis carolinensis* have not been systematically investigated. The scale microstructures across key body regions (head, dorsum, venter, tail, and hindleg) of *A. carolinensis* were meticulously examined using scanning electron microscopy. Statistical analyses were further conducted on the morphological types, density, and distribution of skin sense organs. The scales

were predominantly irregular hexagons, although nearly circular scales were observed on the head. Scale area varied significantly across body regions, with the largest scales located on the tail (0.12 ± 0.02 mm^2). The head and ventral scales were intermediate in size (0.09 ± 0.04 mm^2 and 0.09 ± 0.01 mm^2, respectively), while the dorsal and hindleg scales were the smallest (0.06 ± 0.01 mm^2 and 0.03 ± 0.01 mm^2, respectively). The distribution of cutaneous sensory organs was non-uniform across the body. In females, two morphotypes—hair-like and pillow-like sensory organs—were identified, with the former approximately 20 times more numerous. In contrast, male individuals possess only hair-like sensory organs. Moreover, the total number of these organs in males is less than that in females, accounting for approximately half of the total number of sensory organs in females. These organs, located adjacent to a raised median ridge on each scale, were more densely distributed on the head, venter, hindlegs, and tail (0–4 per scale) compared to the dorsum (0–1 per scale). This study details the adaptive microstructures of scales and sensory organs in *A. carolinensis*, providing key morphological insights into their interactions with the environment. Beyond deepening our knowledge of squamate integumentary adaptations, the work directly contributes to understanding the mechanistic basis of color change and thermoregulation. Furthermore, this study offers valuable insights for the development of novel biomimetic materials, such as adaptive camouflage systems.

5.12. The 5 × FAD Mouse Model: A Translational Tool for Understanding Amyloid Pathology and Cognitive Decline in Alzheimer's Disease

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Animal models with amyloid pathology are fundamental for elucidating the mechanisms underlying Alzheimer's disease (AD). These models can be classified into toxicological, pharmacological, and transgenic categories, depending on how neurodegeneration is induced.

The 5 × FAD (B6SJL) mouse line, carrying five human mutations in *APP* and *PSEN1* (Swedish, Florida, London, M146L, L286V), represents one of the most aggressive and well-characterized transgenic models for early-onset AD. These mice exhibit intraneuronal $A\beta_{42}$ accumulation as early as 1.5 months, followed by extracellular plaque deposition, glial proliferation, and cortical neurodegeneration. At 4–6 months, they display marked synaptic loss (reduced synaptophysin and PSD-95), memory deficits in Y-maze and Morris water maze tests, and altered olfactory and social behaviors. Sex-dependent differences have also been reported, with females showing enhanced *APP* overexpression and accelerated pathology, likely estrogen-modulated. By nine months, cognitive and motor impairments are pronounced, reflecting extensive amyloidosis and neuronal loss across hippocampal and cortical regions.

The 5 × FAD model, thus, integrates key neuropathological, molecular, and behavioral hallmarks of human AD, making it a powerful platform for testing phytochemical compounds and exploring mechanisms of amyloid clearance, neuroinflammation, and neuroprotection. Despite interspecies limitations, its rapid, reproducible pathology continues to provide invaluable insight into the progression and treatment of Alzheimer's disease.

6. Marine Biology

6.1. Population Structure of the European Seabass (Dicentrarchus labrax) in the Atlantic Iberian Peninsula Waters Inferred from Otolith Elemental Signatures

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The European seabass (*Dicentrarchus labrax*) is a key coastal species in the northeast Atlantic, supporting valuable commercial and recreational fisheries. However, within the Iberian divisions 8c–9a, as defined by the International Council for the Exploration of the Sea (ICES), information on stock structure remains scarce. To address this gap, otolith elemental composition (element:Ca ratios) was analysed in 75 pre-selected three-year-old individuals (25 per location) collected between January and March 2025 from three sites along the Atlantic coast of the Iberian Peninsula: Avilés, Peniche, and Lagos. Elemental signatures from sagittal otoliths were obtained using solution-based and laser ablation inductively coupled plasma mass spectrometry (SB-ICP-MS and LA-ICP-MS) for bulk, core, and edge analyses, respectively. Six informative trace elements (Sr, Ba, Mg, Mn, Cu, and Li) were quantified, and spatial variation was assessed using univariate and multivariate statistics. Results revealed moderate spatial variability in otolith chemistry, with low to medium overall reclassification success (52% for bulk, 41% for core, and 56% for edge). Distinct elemental patterns were observed across locations, including elevated Mn:Ca ratios in Avilés, likely linked to local Mn pollution, and higher Li:Ca and Ba:Ca ratios in Peniche and Lagos, probably reflecting upwelling influence. Despite some regional heterogeneity, the overall overlap in otolith chemical signatures supports the ICES definition of a single, though not entirely homogeneous, seabass stock in the Iberian divisions (8c–9a). These results highlight a balance between connectivity and regional differentiation in *D. labrax* populations. Integrating complementary tools such as genetics, tagging, isotopic, and parasite assemblage analyses is recommended to further clarify connectivity and stock dynamics, supporting science-based management and conservation of seabass fisheries.

6.2. Stage-Specific Thermal Sensitivity in Early Development of Tripneustes gratilla (Linneaus 1758)

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The developmental success of marine ectotherms depends heavily on temperature, an important environmental factor, particularly during early life stages that are highly sensitive to thermal stress. In this study, we investigated how *Tripneustes gratilla* embryos developed at different temperatures (20 °C, 25 °C, 28 °C, 30 °C, and 35 °C) throughout their early developmental stages, which include fertilization, cleavage, morulation, blastulation, gastrulation, and larval formation. Cumulative proportion of success over time (CPS, proportion-hour) estimates were fitted against temperature in a non-linear thermal reaction norm curve using the Lactin-2 model variant to determine key thermal traits, including the maximum CPS (CPS_{max}), thermal optimum (T_{opt}), critical thermal limits (CT_{min} and CT_{max}),

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thermal tolerance range (TTR), and thermal safety margin (TSM). Results revealed optimal development between 25 °C and 28 °C, with reduced success and developmental arrest at thermal extremes. Importantly, thermal tolerance narrowed and T_{opt} increased with developmental progression, indicating increased sensitivity in later stages. The research demonstrates that thermal windows exist at specific developmental stages, and the larval stage shows the narrowest tolerance range and safety margin. Our results highlight the vulnerability of *T. gratilla's* early development to ocean warming and underscore the need to identify thermal thresholds to inform conservation strategies for marine invertebrates under climate change.

6.3. Biochemical Changes in Non-Native Pink Salmon Oncorhynchus gorbuscha Spawners Depending on the Timing of Upstream River Movement

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In recent decades, populations of the non-native pink salmon *Oncorhynchus gorbuscha* have successfully expanded their range in the White Sea basin. Understanding the physiological characteristics of pink salmon in these novel aquatic ecosystems is crucial for assessing potential risks to native ichthyofauna, including other salmonid species. Timing of pink salmon entry from the sea into the rivers often varies during spawning migration. We selected two sampling time points of the species entry into the Keret River after crossing the critical salinity barrier from the sea to the river: in mid-July (the peak) and one and a half months later, at the end of August (termination/end). To evaluate steroidogenesis and energy exchange in fish, we measured the individual concentrations of sex steroids and thyroid hormones in the blood serum. Additionally, levels of glucose and cortisol were quantified to assess fish osmotic stress after crossing the critical salinity barrier (from the sea to the river). We observed that larger males and females predominantly migrated to the river at the end of the spawning season. During migration termination, both sexes exhibited a four- to five-fold decrease in estradiol-17β concentrations compared to fish that had passed upstream earlier, indicating the completeness of gonad development. It appears that gonad development in later spawners nearly completes in brackish water. The observed reduction in triiodothyronine levels by three to four times from July to August suggests differing energy mobilization and utilization processes between the two groups of spawners. Earlier and later spawners also differed in their stress responses, as the former exhibited lower levels of cortisol and glucose than the latter. Such biochemical heterogeneity likely characterizes the successful adaptation of non-native fish to new habitats, which is a critical factor that needs to be considered when assessing their impact on aquatic ecosystems.

6.4. Biological Succession and Degradation in Biofouled Plastics

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Plastic pollution in marine environments is a growing ecological issue, yet the role of biofouling (biological colonisation of plastic surfaces) remains insufficiently understood, particularly with regard to its effects on plastic degradation and transport. This study examined how biofouling alters expanded PVC debris over time, both biologically and physically, through an in situ experiment conducted at the Marina of Oeiras, Portugal. Circular PVC slabs were submerged and retrieved after 7, 30, 60, and 180 days. Colonisation

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was assessed via microscopy DNA-based microbial identification using Sanger sequencing and dry-weight measurements.

Over the exposure period, clear ecological succession was observed: early colonisers such as *Skeletonema pseudocostatum* and *Minidiscus spinulatus* (diatoms) gave way to more diverse communities, including *Nannochloropsis oculata* and *Melosira tropica* and invertebrates like *Watersipora subtorquata* and *Balanus trigonus*. This biological progression reflected a maturing biofilm that likely influenced both the microbial composition and the material properties of the plastic.

Microbial analyses identified several plastic-associated species with known biodegradative capabilities, including *Pseudomonas hunanensis*, *Comamonas testosteroni*, and *Fusarium* spp., indicating potential early-stage microbial degradation. The presence of faecal indicator bacteria such as *Escherichia coli* and *Enterobacter* sp. indicated anthropogenic influence, suggesting that human-derived nutrients may shape plastisphere communities.

These biological changes were paralleled by physical alterations: disc weight increased steadily with time and depth, consistent with biomass accumulation.

Overall, marine biofouling drives significant biological and physical changes in plastic debris, with early evidence of microbial degradation. These interactions could influence the long-term fate, transport, and persistence of plastics in the ocean and should be integrated into marine plastic pollution models.

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6.5. Histology-Based Circulatory System Reconstruction of Sepiolidae (Bobtail Squids) and Renal Parasite Documentation

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The circulatory system of cuttlefish (Sepiidae) has been extensively described, yet no comprehensive reconstruction exists for bobtail squids (Sepiolidae), a family of ecological and evolutionary importance within Decapodiformes. To address this gap, we examined five cuttlefish species (*Sepia lycidas, S. esculenta, S. tenuipes, S. japonica, and S. kobiensis*) and two bobtail squids (*Rossia bipapillata and Sepiolina neponensis*). For each specimen, morphometric parameters (sex, total length, and mantle length) were recorded, and branchial hearts and renal appendages were processed for histological analysis using serial microtomy and a panel of stains (H&E, PAS, Masson's trichrome, and Giemsa). This approach allowed us to trace vessel continuity, characterize tissue architecture, and identify sites of parasite colonization. From these observations, we established and illustrated the **first complete circulatory system diagram for Sepiolidae**, providing histological confirmation of vascular connections between gills, branchial hearts, systemic heart, and renal appendages. While the gross architecture parallels that of Sepiidae, sepiolids exhibited distinctive renal appendage histology, including denser epithelial folding and variable vascularization. Furthermore, renal tissues were screened for parasitic taxa, revealing the presence of dicyemids and *Chromidina* spp. in select individuals. Comparative histology has previously shown that the external surface of renal organs varies morphologically across cephalopods [1], and one dicyemid species was found in the branchial heart appendage of *Rossia pacifica* [2]. By combining morphometrics, histology, and parasitology, this study delivers the first validated account of sepiolid circulation, closes a major anatomical knowledge gap in Decapodiformes, and establishes a framework for future research on cephalopod physiology, host–parasite interactions, and evolutionary adaptations of the circulatory system.

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6.6. Impacts of Morbillivirus Epizootics on the Abundance and Population Structure of the Striped Dolphin (Stenella coeruleoalba) in the Western Mediterranean

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In the Mediterranean Sea, the dolphin morbillivirus caused severe mortality among striped dolphins (*Stenella coeruleoalba*) during 1990–1992 and 2007, although the latter outbreak appeared to be less intense. In response to these events, this study evaluated whether epizootics led to the stranding of typically offshore individuals that would not have been stranded during non-epizootic periods, and how these episodes affected population abundance and age structure. To address the first objective, morphometric data recorded in necropsy reports of stranded dolphins between 1980 and 2024—compiled by the University of Valencia—were analyzed using principal component analysis. To assess the second objective, temporal patterns of stranding frequency and the relative proportions of age classes were examined. Results indicated that dolphins stranded in 1990 exhibited significantly larger multivariate body sizes compared to other years, except for the 1980–1989 period. Regarding morphometry, the principal component most strongly associated with fin measurements revealed that 1990 differed from all periods except 2007, which, in turn, only differed from the inter-epizootic interval. The number of strandings did not decline following epizootics; rather, it increased and stabilized around 2013. Nonetheless, after 1990, the age structure became dominated by calves and juveniles. These findings suggest that during epizootics, particularly that of 1990, individuals exhibiting oceanic morphometric traits may have been stranded, and selective adult mortality may have altered the population's age composition. Although striped dolphins displayed a distinct trend in stranding frequency compared to other cetaceans, disentangling the effect of the epizootic from that of the establishment of the stranding network in 1990 remains challenging. Overall, the results highlight the potential population-level consequences of epizootics in long-lived species and emphasize the importance of maintaining effective stranding monitoring networks.

6.7. Marine Life in a Changing Ocean: Biological Impacts and Adaptive Responses to Climate Stressors

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Climate change is forcing unprecedented changes in ocean temperature, chemistry, and circulation, with profound implications for marine life and ecosystems. This review consolidates existing knowledge of how major climate stressors—ocean warming, acidification, deoxygenation, and sea-level rise—impact marine biology across levels of organization. We investigate physiological disturbances in keystone species, for example, coral bleaching, shifted metabolism in fish, and compromised calcification in mollusks and plankton. Particular emphasis is placed on shifts in reproduction cycles, migration patterns, and species distribution, which are already reorganizing food webs and ecosystem processes. The review also draws on novel genomic and ecological evidence of adaptive responses, such as thermal tolerance, phenotypic plasticity, and microbial symbiosis. Despite local examples of resilience, many marine organisms are at risk of increased threats of extinction, particularly in tropical and polar systems. We identify major knowledge gaps in multi-stressor research, long-term monitoring of ecosystems, and predictive modeling under high-emissions projections. By integrating data from molecular biology, marine ecology, and climate science, this review aims to guide conservation management practice, marine protected area planning, and sustainable fishery management. Understanding the physiological responses of marine organisms to climate change is not only critical for maintaining biodiversity, but also for the health and stability of global ocean systems that provide human livelihoods.

6.8. Marine Mugil Cephalus Gut-Derived Pseudomonas stutzeri GM30 from: A Novel Probiotic and Amylase-Based Postbiotic for Sustainable Aquaculture

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Asian aquaculture faces growing threats from biofilm-forming bacterial pathogens, compounded by widespread antimicrobial resistance from antibiotic overuse. This study explores host-derived probiotics as viable alternatives by isolating gut bacteria from *Mugil cephalus*, a key marine fish species cultured along India's southeastern coast. Among 28 isolates recovered from intestinal contents, strain GM30 showed promising antimicrobial and enzymatic properties. Molecular identification using 16S rRNA gene sequencing confirmed it as *Pseudomonas stutzeri*, showing 99% similarity Using agar well diffusion assays, GM30 demonstrated inhibitory activity against six common fish pathogens, including *Aeromonas salmonicida*, *Aeromonas caviae*, *Vibrio harveyi*, *Vibrio vulnificus*, *Edwardsiella tarda*, and *Staphylococcus aureus*. The strain exhibited favourable probiotic characteristics: 37.1% surface hydrophobicity, indicating high adhesion potential; 3% tolerance to bile salts; 70.5% autoaggregation capacity after 24 h; and 29.03% antioxidant activity via DPPH radical scavenging. GM30 produced several digestive enzymes: amylase (0.68 U/mL), pectinase (0.93 U/mL), protease, lipase, and cellulase, which could improve nutrient utilisation and feed conversion efficiency in commercial settings. Interestingly, the partially purified amylase fraction (50–65 kDa) showed activity beyond simple starch breakdown. It appeared to disrupt the polysaccharide structures within pathogenic biofilms, suggesting an additional protective mechanism against persistent infections. This combination of pathogen inhibition, enzyme production, gastrointestinal stress tolerance, and potential biofilm disruption makes *P. stutzeri* GM30 worth investigating further for practical aquaculture applications. The results support developing antibiotic-free strategies that address both productivity en-

hancement and disease management challenges in intensive fish farming systems prevalent across Asia.

6.9. Microplastic Contamination in Anchovies (Engraulidae) Sold in Mercedes Fish Port in Camarines Norte, Philippines

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Marine organisms, including anchovies, are known to ingest microplastics—plastic particles less than 5 mm in diameter, raising significant ecological and human health concerns. This study examined the presence, composition, and potential sources of microplastics in anchovies collected from the Mercedes Fish Port in Camarines Norte, Philippines. A descriptive research design was employed, with 64 anchovy samples selected randomly for analysis. The samples underwent whole-body digestion, saline solution flotation, filtration, visual inspection, dimensional measurement, and Fourier Transform Infrared (FTIR) analysis. All recovered microplastics were identified as fibers with exclusively filamentous shapes. Among the colored plastic particles, red (50%) was the most prevalent, followed by black (25%) and pink (25%). These microplastic fibers varied in size, ranging from 201–400 μm, to 601–800 μm, to over 1000 μm. Fourier Transform Infrared (FTIR) analysis confirmed that all microplastic fibers were made of polyester, a common synthetic polymer. These findings suggest that textile waste and fishing-related activities are the likely sources of microplastic contamination in anchovies. The predominance of red-colored microplastic fibers supports a potential link to dyed synthetic materials commonly found in textile products and fishing gear. Microplastics were detected in 6.25% of anchovy samples from the Mercedes Fish Port—marking the first documented case of microplastic ingestion by anchovies in the Bicol Region. Although the occurrence rate is low, the presence of synthetic polyester fibers signals an emerging threat to marine ecosystems and food safety. This finding underscores the urgent need for increased awareness and proactive steps to address microplastic pollution. Given the serious implications to aquatic life and potential risks to human consumers, continued monitoring of microplastic contamination in marine species is highly recommended. Further research is crucial to evaluate the long-term ecological impacts, dispersal patterns, and potential health risks associated with microplastic contamination in marine organisms and human consumers.

6.10. Otolith Microchemistry to Assess the Habitat Use of Atherinella brasiliensis in Two Estuaries with Distinct Salinity Ranges in Northeastern Brazil

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The estuarine ecosystems are driven by pronounced gradients and dynamic processes, leading to high spatial and temporal variability in both biotic communities and abiotic conditions. This study examines the effect of a range of salinities on the chemical composition of the sagittal otoliths of Brazilian silversides *Atherinella brasiliensis* from two tropical estuaries (positive and hypersaline). Beach seine sampling was conducted in the upper and lower zones of the estuaries during both the rainy (June, August 2016) and dry seasons (October 2016, January 2017). Multi-elemental signatures (MESs: Element:Ca)

of the bulk otoliths were obtained using solution-based inductively coupled-plasma mass spectrometry (SB-ICP-MS). To determine the spatial variation in otolith signatures, we analyzed patterns of homogeneity and heterogeneity between estuaries using univariate and multivariate statistics. Overall, 121 otoliths were analyzed from adult fish collected in both estuaries. The data indicated significant differences in the elemental composition between individuals collected in positive and hypersaline estuaries. Elemental differences were mainly driven by Sr and Zn in the hypersaline estuary, while Ba and Mg were higher in the positive estuary. The MES compositions were correctly reclassified into their original groups in each zone from both estuaries: 86.7% and 83.3% in the positive estuary and 66.7% and 56.7% in the hypersaline estuary, for inferior and superior zones, respectively. The overall reclassification rate obtained using MESs was 73.3% for both estuaries. The salinity gradient in estuaries is the most influential habitat factor affecting species distributions, and the variation in elemental concentrations across the depositional time frame of the otolith reflects the exposure of fish to different salinity ranges.

6.11. Population Dynamics, Growth, and Reproductive Phenology of Metapenaeus moyebi in a Tropical Lagoon

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Metapenaeus moyebi, a lacustrine penaeid shrimp exhibiting complete estuarine residency, constitutes a major component of tropical lagoon shrimp fisheries. This study provides the first comprehensive population assessment of the species in Sri Lankan waters, based on monthly sampling of 4200 individuals from the Jaffna Lagoon between January 2022 and January 2025. Population parameters were estimated using TropFishR, and length at 50% sexual maturity (L_{m50}) was analyzed using SizeMat. Spatial and temporal patterns were examined through distributional and phenological analyses. The von Bertalanffy growth parameters were estimated as $L\infty$ = 11.2 cm, K = 2.64 yr^{-1}, and t_{anchor} = 0.18 yr. Estimated fishing mortality (F = 7.54 yr^{-1}), was below the maximum exploitation threshold (F_{max} = 8.91 yr^{-1}), with F/F_{max} = 0.73. However, ratios relative to conservative reference points, $F/F_{0.5}$ (2.64) and $F/F_{0.1}$ (1.71) exceeded 1, indicating potential sustainability concerns. Length at 50% and 75% capture (L_{c50} = 5.98 cm, L_{c75} = 6.27 cm) were smaller than L_{m50} (7.4 cm), suggesting growth overfishing due to harvest of individuals before sexual maturity. Reproductive phenology showed peak maturity of females (94%) by November, indicative of the spawning season, while immature individuals dominated catches from March to May, reflecting the main recruitment period into the lagoon population. Spatial analysis revealed significant ontogenetic segregation (p 0.05), with immature shrimp concentrated near the lagoon mouth (52%) and decreasing toward inner lagoon zones. These findings demonstrate that *M. moyebi* maintains a resident life cycle with distinct spatial and temporal population structuring. Sustainable management requires spatially explicit conservation measures, including protection of nursery habitats and seasonal harvest closures to maintain stock resilience.

6.12. Population Structure of the Brazilian Sardine Sardinella Brasiliensis Inferred from Age-Based Otolith Growth Rates

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This study investigates the population structure of the Brazilian sardine, *Sardinella brasiliensis*, Brazil's primary fishery resource, by analyzing growth rates across its geographical distribution. Historically, the population is divided into a northern (22–24 °S) and a southern stock (26–29 °S), with a central area (25 °S) hypothesized as a shared juvenile feeding ground. Individuals are believed to return to their natal stocks upon reaching sexual maturity. To test this long-standing hypothesis, this research compared the growth rates of 50 sardines, aged 2 to 7 years, from the northern, central, and southern areas. The methodology involved measuring the average distance between growth rings on otoliths (ear stones) and analyzing these distances using a generalized additive model for scale and shape, followed by Dunn's tests for multiple comparisons. The analysis revealed significant differences in growth patterns at specific life stages. For the earliest life stages (first and second post-nuclear rings), growth in the central area was distinct, supporting its role as a shared nursery ground. At ages 1 and 2, significant differences emerged between the southern area and both the northern and central areas but not between the north and central regions, indicating a mixing of stocks. By age 4, differences were found solely between the established northern and southern stocks, with no distinction between the central area and either stock. These growth rate findings robustly corroborate previous studies using geometric body shape and otolith chemistry. The results confirm the historical population model: juveniles from both stocks cohabitate and feed in the central area before returning to their respective northern or southern regions around their fourth year of life. This research provides critical evidence for the species' population dynamics, offering vital insights for its sustainable management and conservation.

6.13. Temperature Dependence of Toxin Production in a Dinoflagellate Strain

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Ocean temperatures are increasing at a critical rate, altering the geographic distribution and likely the frequency and severity of toxic algal blooms. Hence, understanding how temperature influences toxin production in toxic algal species is critical for predicting future toxic bloom dynamics under climate change scenarios. In this study, we investigated the temperature dependence of the production of okadaic acid (OA) and dinophysistoxins (DTX1 and DTX2) in the marine dinoflagellate *Prorocentrum lima* CCAP 1136/11. Experiments were conducted to determine toxin production under rapid and gradual exposure to a temperature gradient from 5 °C to 25 °C in 5 °C increments. LC-MS/MS analysis confirmed the presence of OA, DTX1, and DTX2, suggesting toxigenicity of this dinoflagellate strain. Temperature had a significant effect on the cellular content, relative composition, and cellular production rates of OA and DTX1. Rapid temperature changes resulted in higher OA/DTX1 ratios compared to gradual exposure, highlighting the complex temperature-dependent dynamics of toxin production. Furthermore, these findings support the hypothesis that toxin production is a stress response mechanism, uncoupled from growth, and is triggered by suboptimal temperatures. The results underscore the

importance of incorporating acclimation effects in models forecasting harmful algal bloom toxicity, particularly in the context of increasing thermal variability under climate change.

7. Plant Biology

7.1. Impact of Drought and Elevated CO_2 on Maize Photosynthesis and Yield in Northern Ghana

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Maize is a staple crop in Northern Ghana, where climate change is increasing the frequency and severity of droughts. Understanding how elevated atmospheric CO_2 interacts with water limitation is critical for improving crop productivity and food security. This study evaluated the effects of drought and elevated CO_2 on maize photosynthesis, stomatal conductance, and yield under field conditions.

Maize plants were grown under ambient (≈410 ppm) and elevated CO_2 (≈600 ppm) conditions, with well-watered and drought-stressed treatments. Leaf-level gas exchange and biochemical assays were used to measure photosynthetic rate, Rubisco activity, and stomatal conductance. Canopy-level carbon fluxes were monitored using portable flux chambers.

Results showed that drought reduced net photosynthesis by 42% (from 22.5 ± 1.2 to 13.0 ± 0.8 μmol CO_2 m^{-2} s^{-1}) and stomatal conductance by 55% (from 0.35 ± 0.02 to 0.16 ± 0.01 mol H_2O m^{-2} s^{-1}) under ambient CO_2. Elevated CO_2 partially mitigated these effects, increasing net photosynthesis by 28% under drought conditions and reducing stomatal closure by 15%. Canopy-level measurements indicated that total aboveground biomass decreased by 38% under drought at ambient CO_2, but only 22% under elevated CO_2.

These findings indicate that elevated CO_2 can partially offset drought-induced declines in maize productivity, but significant reductions still occur under severe water limitation. Breeding and management strategies that enhance drought tolerance while optimizing CO_2 responsiveness could improve maize yield stability in Ghana's increasingly variable climate.

7.2. Can Artificial Plantings Resemble Natural Vegetation? Preliminary Evidence

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The Nature Restoration Law approved by the EU Commission in 2024 commits Member States to restore 30% of degraded habitats by 2030. The achievement of this ambitious goal poses several challenges, including the ability to predict the outcome of artificial interventions and to what extent they can restore or initiate dynamics leading to ecosystems that become stable over time and are similar to those resulting from natural evolutionary processes. The lack, or fragmentation, of historical data concerning past land use and human interventions greatly reduces our understanding of the legacy of such activities.

A plantation of *Quercus robur* L. (English oak) mixed with *Fraxinus angustifolia* Vahl (narrow-leaved ash) set up in 1985 in an area used for centuries as pasture and then for farmland and bordering on an old-growth forest classified as *Fraxino-Quercetum roboris*, Gellini et al. 1986, gave us the opportunity to compare understorey vegetation originating from long-term natural forest dynamics with that derived from secondary colonization after treefall. The understorey vegetation was analysed for composition and abundance in plots arranged in mature forest stands (MF), in natural forest gaps (FG), and within the artificial plantation (AF). Species richness and cover/abundance following Braun-Blanquet were determined on five 400-square-metre plots for each vegetation type. The occurrence

of species in 16 quadrats (50 × 50 cm) set along the plot diagonals was used to estimate vegetation diversity.

After forty years, AF shared 60% of species with MF and was populated by typical nemoral species. Species richness was almost double in FG than in forests, but the proportion of alien species was 7% compared to 2.5% in MF and 0% in AF.

These results provide valuable insights into the ability of artificial forest plantations to restore natural habitats, provided that connectivity with natural systems is maintained to ensure the supply of forest species propagules.

7.3. Exploring the Potential of Rice Wash to Promote Germination in Eggplant (Solanum melongena)

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Eggplant (*Solanum melongena* L.) is a high-value crop, but its seeds often exhibit uneven and delayed germination. To address this, the use of priming agents that promote uniform, rapid germination and enhance seed vigor is being investigated. Rice wash (RW), locally known as *hugas bigas*, is a common household by-product derived from rinsing rice grains. Anecdotal reports suggest that when used for watering ornamentals, RW promotes better plant growth and flowering. This study aimed to evaluate the potential of RW from different rice cultivars as a seed-priming agent to improve the germination and vigor of eggplant seeds. Seeds were soaked in RW for 12 h, air-dried for another 12 h, and germinated on moist filter paper using the top-of-paper method. Daily germination counts were recorded, and germination rate (GR), vigor index (VI), and seedling dry weight (SDW) were determined. Treatments were arranged in a Completely Randomized Design (CRD) with three replications. Data were analyzed using ANOVA, and means were compared using LSD at $\alpha = 0.05$. Results showed that seeds soaked in white rice wash (WRW), red rice wash (RRW), and brown rice wash (BRW) exhibited earlier germination—observed as early as one day after sowing—and higher germination percentages. In terms of vigor, WRW and RRW treatments resulted in more uniform and faster germination than other rice wash types. Seedling dry weights at seven days after sowing were comparable across treatments. In conclusion, WRW, RRW, and BRW enhanced early, uniform, and rapid germination of eggplant seeds.

7.4. In Vivo Assessment of Rosmarinus Officinalis and Olea Europaea Leaf Extracts Enriched in Rosmarinic Acid and Oleuropein as Modulators of Metabolic Inflammation

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Plant-derived phenolic metabolites structurally related to phytohormone signaling molecules have gained interest for their potential to modulate oxidative stress and inflammation associated with metabolic disorders. *Rosmarinus officinalis* (rosemary) and *Olea europaea* (olive) leaves contain bioactive compounds such as rosmarinic acid and oleuropein,

which are involved in plant defense and signaling pathways and may influence inflammatory processes in mammals. This study aimed to investigate the in vivo antioxidant and anti-inflammatory effects of these metabolites. Methanolic extracts of *R. officinalis* and *O. europaea* leaves were prepared and administered orally to adult male Wistar rats (8–10 weeks, 200–250 g; n = 6 per group) at 200 mg/kg/day for 28 days. Rats were assigned to control, high-fat diet (HFD), and HFD + plant extract groups. Extracts were characterized by HPLC, confirming rosmarinic acid (12.5 mg/g extract) and oleuropein (8.3 mg/g extract). Serum lipid profiles, hepatic oxidative markers (MDA, SOD, CAT), and inflammatory cytokines (TNF-α, IL-6) were quantified. Treatment with R. officinalis and O. europaea extracts significantly reduced serum triglycerides (−28%) and total cholesterol (−31%) compared with HFD rats. Hepatic MDA decreased by 36%, while SOD and CAT activities increased by 41% and 38%, respectively. NF-α and IL-6 levels were markedly downregulated, indicating attenuation of metabolic inflammation. These results demonstrate that phenolic metabolites structurally related to phytohormone signaling molecules exert potent antioxidant and anti-inflammatory effects in vivo, highlighting their potential as natural modulators of metabolic inflammation and contributors to metabolic homeostasis.

7.5. A Telomere-to-Telomere Genome Assembly Provides Insight into Glucosinolate Biosynthesis in Descurainia sophia

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Descurainia sophia Willd. belongs to the genus *Descurainia* of Cruciferae. Its dried, mature seeds are known as "Tinglizi" and are widely used in traditional Chinese medicine. The seeds of *D. sophia* are rich in unsaturated fatty acids, glucosinolates, flavonoids, and a variety of compounds with biological activity. In order to obtain detailed information on the genome of *D. sophia* and to explore the molecular basis of glucosinolate biosynthesis in *D. sophia*. We assembled a high-quality telomere-to-telomere (T2T) reference genome of the *D. sophia* genome (2n = 4x = 28) using PacBio and ONT long-reads, in combination with Illumina short-read sequencing and Hi-C technology. The assembled genome was 266.23 Mb in size, with 36.12% GC content and 23.28% repeat content. There are 14 pseudo-chromosomes in the Hi-C-assembled genome sequence. A total of 57,590 genes and 87 pseudogenes were predicted, 98.94% of which can be annotated to Nr, GO, KEGG, TrEMBL, Swissport, Pfam, and InterPro databases. Comparative genomic analyses indicated that the expanded gene families were significantly involved in sesquiterpenoid and triterpenoid biosynthesis, homologous recombination, and inositol phosphate metabolism. Moreover, we identified 55 genes involved in glucosinolate biosynthesis in *D. sophia*. UGT74B1 and SOT16 catalyze the final two steps of glucosinolate biosynthesis; we cloned these genes and verified their functions using in vitro catalysis and transgenic *A. thaliana*. Over-expression of the *DsUGT74B1* gene can significantly promote the growth of *A. thaliana* by regulating IAA and BR signal pathways, and the content of glucosinolates and flavonoids in the transgenic plants was remarkably higher than that in the wild-type *A. thaliana*. The high-resolution reference genome generated by this study will provide accurate sequence information for glucosinolate biosynthesis research of *D. sophia*.

7.6. Adaptive Mechanisms of Cereal Crops Facing Combined Abiotic Stresses

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Cereal crops are increasingly exposed to multiple abiotic stresses. These stresses are drought, salinity, and extreme temperatures, and they have a negative impact on global

food security. Recent research studies show that root system architecture (RSA) plays an significant role in stress resilience. However, there is still a lack of studies on the effect of combined abiotic stresses on the adaptive mechanisms of roots. In this review, key physiological and genetic responses that enhance tolerance will be illustrated in oats, barley, and wheat. In some barley genotypes (e.g., Barke), drought and heat caused the development of deeper and steeper axial roots, necessary for sustained water uptake and the conservation of resources from the soil. In contrast, wheat lines possessing the HKT;5 allele exhibited superior Na+ exclusion under combined salinity and drought conditions. These conditions also enhanced aerenchyma formation, causing metabolic costs to become reduced and root penetration in compacted (or saline) soils to become improved. In oats, on the other hand, waterlogging and salinity induced adventitious roots and sclerenchyma formation. This improved oxygen supply, as well as reducing ion toxicity in oats. At the molecular level, it has been reported that NAC and WRKY transcription factors, together with PIN1 and PIN3 auxin transporters, regulate shifts in root growth angle, branching density, and cortical cell differentiation in response to multiple environmental cues. The fact that plants use unique developmental programs is reinforced by modern imaging platforms (e.g., X ray CT, rhizotrons, and shovelomics), which suggest that combined stresses trigger non-additive RSA responses, thereby requiring multiple-stress studies. Therefore, in this review, I will provide a framework for breeding climate-resilient cereal crops, such as oats and barley, in the context of changing environmental and climate conditions.

7.7. Advances in Genome-Wide Association Studies for B-Glucan Content in Barley (Hordeum Vulgare L.): Review

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Unraveling the genetic architecture of complex traits remains a major challenge in plant genomics. Genome-wide association studies (GWAS) represent a powerful approach for dissecting the genetic basis of complex quantitative traits, relying on advanced statistical models to identify candidate genes associated with phenotypic variation. In this work, we review advances in understanding the genetic control of β-glucan content in barley, a key trait with significant implications for human health and the food industry. We first provide an overview of GWAS as a tool, explaining how it works, why it is powerful, and how different statistical models such as the General Linear Model (GLM), the Mixed Linear Model (MLM), and other approaches help to reduce false positives and false negatives in marker–trait associations. By synthesizing results from previous GWAS studies, we highlight the major genomic regions and candidate genes associated with β-glucan content. We further discuss the added value of GWAS for genomic selection, where the identification of informative loci can guide the selection of superior individuals for breeding. Additionally, we emphasize the importance of integrating bioinformatics tools. This work provides an updated perspective on β-glucan genetics and the associated methodologies, offering valuable insights for future breeding strategies to enhance the nutritional quality of barley.

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7.8. Arati-Less Tumor: In Vivo Tumoricidal Activity of Aratiles (Muntingia calabura L.) Leaves Ethanolic Extract with Chitosan Derived from Dalagang Bukid (Caesio cuning) Against UVB-Induced Tumor in Fruit Flies (Drosophila melanogaster Meigen)

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Malignancy remains one of the leading causes of mortality worldwide, with global cases projected to exceed 35 million by 2050. Conventional chemotherapeutic agents such as doxorubicin, though effective, are often costly and accompanied by severe toxic effects, underscoring the need for safer, natural, and affordable alternatives. This experimental and quantitative study evaluated the tumoricidal activity of Aratiles (*Muntingia calabura* L.) leaves ethanolic extract stabilized with chitosan derived from Dalagang Bukid (*Caesio cuning*) against UVB-induced tumors in Fruit flies (*Drosophila melanogaster Meigen*). The ethanolic extract was obtained through maceration, while chitosan was isolated from fish scales through deproteinization, demineralization, and deacetylation. Tumor-bearing fruit flies were grouped into 25%, 50%, and 75% extract concentrations, alongside positive (doxorubicin) and negative (distilled water) controls. Data were gathered through microscopic and behavioral observations, focusing on tumor regression, mortality, and overall flies' vitality. Phytochemical screening confirmed the presence of alkaloids, flavonoids, and triterpenoids such as lupenone, compounds known for their tumor-suppressive properties. Results showed that the 50% extract concentration exhibited the highest tumor regression (51.86%), comparable to doxorubicin (54.37%) but with reduced mortality (66.67% vs. 100%). Statistical analysis using one-way ANOVA revealed a significant difference in tumor size reduction ($F = 76.12$, $p < 0.001$) but no significant difference in mortality ($p = 0.392$). These findings suggest that the synergistic effects of lupenone-rich triterpenoids, alkaloids, flavonoids, and chitosan enhanced tumor regression while minimizing toxicity. Overall, the study concludes that Aratiles ethanolic leaf extract with chitosan shows promising potential as a sustainable, safe, and low-cost alternative to conventional chemotherapeutics, meriting further validation in mammalian models for clinical application.

7.9. Comprehensive Study of Leaves of Some Bamboo Species Showcasing Their Anatomical Differences

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In bamboos, leaves are central not only to plant physiology but also to taxonomic study, since macromorphological characteristics such as culm or branching often overlap among species. Leaf anatomy therefore provides a valuable lens for understanding how bamboo functions and for resolving its often-challenging classification. This study focused on finding out the anatomical differences among the leaves of four bamboo species, *Bambusa longispiculata*, *Bambusa ventricosa*, *Bambusa multiplex*, and *Dendrocalamus giganteus*. The plant leaves were collected from Botanical Garden of Rajshahi University, Rajshahi, Bangladesh. The surface of the leaf blade was carefully peeled, and stomatal characteristics were observed microscopically. For microscopic structural analysis, slides were prepared from the leaf blade section. The radial extent of the cell was measured utilizing image J softwere. The leaf length, width, and area significantly varied within the bamboo species studied. The stomatal index in *B. ventricosa* was the highest in percentage (17.39%) and lowest in the species *D. giganteus* (6.9%). A variation was found in the arrangement and layer of

the epidermal cell between the costal and intercoastal zones. In the transverse section, the largest diameter was found in the fusoid cell (147.36 ± 23.19 um) in the midst of other cells, i.e., the Meta xylem, proto xylem, phloem, mesotome, bundle sheath, mesophyll, and bulliform. Among the species, *B. longispiculata* had the largest cell diameter and *B. multiplex* had the smallest cell diameter. Variations were found in the number and arrangement of vascular bundles, as well as in the diameters of the mesophyll and bundle sheath cells, as well as other cells among the studied species. These findings demonstrate the importance of anatomical study in understanding bamboo species' physiology, ecological strategies, and functional adaptations. The findings also undercore the interspecific variation among the species.

7.10. Early and Middle Stage Glycation Reversing Activities of Whole Grains of Selected Wild Oryza Species in Sri Lanka

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Wild rice relatives from the genus *Oryza* offer great potential for improving cultivated rice. Although Sri Lanka is home to five wild *Oryza* species, their health-promoting properties have been scarcely studied. Our recent research demonstrated that all five wild rice species available in the country have anti-glycation activity. Building on these findings, the present study aimed to investigate the **in vitro glycation-reversing potential** of two selected species, which showed high anti-glycation activity in our previous studies on the management of diabetes complications. *Oryza nivara* and *Oryza eichingeri* were used in the present study. These two species were collected from their natural habitats, Chenkaladi (Batticaloa) and Waulpane (Embilipitiya), respectively. Freeze-dried 70% ethanolic extracts of whole grains were initially evaluated for early-stage (BSA-Glucose) glycation reversing (n = 3 each) activity. The middle stage glycation reversing activity (BSA-Methylglyoxal:MGO) was subsequently assessed for the species that exhibited the highest anti-glycation and antioxidant properties in our previous studies. Results showed that both species exhibited dose-dependent early-stage glycation-reversing activity, with no significant difference between them. The EC_{50} values of *Oryza nivara* and *Oryza eichingeri were* 36.61 ± 1.26 and 39.61 ± 1.22 µg/mL, respectively. Furthermore, *Oryza eichingeri* shows middle-stage glycation-reversing activity (EC_{50}: 173.92 ± 9.29 µg/mL). In conclusion, both species exhibited notable glycation-reversing activity, suggesting their potential for the management of diabetes-related complications.

7.11. Effects of Two Biochar Soil Amendments and Application Rates on Decreasing NH_4^+ Concentration in Soil to Mitigate NH_4^+ Toxicity and Improve Growth of Canola (Brassica napus L.)

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Introduction

Biochar is a promising soil amendment that can enhance carbon sequestration, improve nutrient availability, and increase crop yield. To evaluate its potential in mitigating ammonium (NH_4^+) toxicity, we conducted a glasshouse trial and a soil incubation study using different rates of oil mallee and wheat chaff biochars that were fully characterised prior to the experiments.

Methods

Two experiments were conducted: a glasshouse trial and a soil incubation study. In the glasshouse experiment, we examined the effects of biochar type (oil mallee, wheat chaff), application rate (0, 5, 10, 20 t/ha), and NH_4^+ supply (15, 60, 120 mg N/kg soil) on canola growth and soil pH.

In the soil incubation experiment, we evaluated the effects of biochar type, application rate (0, 5, 20 t/ha), and NH_4^+ concentration (60, 120 mg N/kg soil) on soil NH_4^+, NO_3^-, total N, pH, and CO_2 emissions. Nitrogen was applied as ammonium chloride (NH_4Cl) in all treatments. Different NH_4^+ levels were used to match the objectives of each experiment; the greenhouse study required a broader toxicity range, whereas the incubation focused on soil N transformations.

Results

In the glasshouse experiment, both biochar types improved canola shoot and root growth. Oil mallee biochar at 10–20 t/ha most effectively alleviated NH_4^+ toxicity symptoms, and at 20 t/ha, increased soil pH ($CaCl_2$) significantly from 5.8 to 6.9.

In the incubation experiment, 20 t/ha oil mallee biochar consistently decreased soil NH_4^+ concentration and promoted a greater shoot accumulation of NO_3^- and total N compared with wheat chaff biochar. The effects on soil pH and CO_2 emissions varied depending on biochar type, application rate, and NH_4^+ concentration.

Conclusion

These results demonstrate that biochar application can substantially improve canola growth and soil chemical properties. Oil mallee biochar is highly effective in mitigating NH_4^+ toxicity in canola.

7.12. Evaluation of Lentil (Lens culinaris Medik.) Genotypes for Yield Performance and Stability Under Rainfed Conditions in Morocco

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Introduction: Lentil (*Lens culinaris* Medik.) is a nutritionally rich legume widely cultivated across diverse agro-ecological zones. In Morocco, it is mainly grown by smallholder farmers under rainfed conditions on limited areas. The grain yield of lentil is strongly influenced by environmental variability, leading to genotype-by-environment interactions (GEI) that slow breeding progress. Understanding GEI interactions is critical for identifying high-yielding and stable genotypes to improve productivity. **Methods:** 40 lentil

genotypes were evaluated across multiple field trials in major lentil-growing regions of Morocco. Trials were conducted under representative rainfed conditions with limited inputs. Grain yield data were collected, and the effects of genotype, environment, and their interaction were analyzed using appropriate statistical models to assess performance and stability. **Results**: Significant effects of genotype, environment, and GEI were observed for grain yield, indicating differential genotype performance across environments. Some genotypes exhibited consistently high yield and stability across environments, whereas others were more sensitive to environmental variation. The analysis allowed the identification of genotypes combining high productivity with broad adaptation. **Conclusions:** The study highlighted the importance of considering GEI interactions in lentil breeding under Moroccan conditions. High-performing and stable genotypes identified here represent promising candidates for enhancing lentil yield and supporting sustainable production. These findings provide valuable insights to increase genetic gain and guide future lentil breeding strategies in Morocco.

7.13. Foliar Application of Folic Acid in Modulation of Growth and Biochemical Attributes of Garlic (Allium sativum L.) Under Salt Stress

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Salinity is one of the major abiotic stresses limiting crop productivity worldwide. According to the Food and Agriculture Organization (FAO, 2021), approximately **833 million hectares of soil** are affected by salinity globally. A pot experiment (3 salinity levels × 4 folic acid concentrations; 3 replicates) was carried out at the Botanical Garden, University of Education, Vehari, to test whether foliar folic acid (FA; 0, 0.2, 0.4, 0.6 mM) improves garlic performance under 0, 50 and 100 mM NaCl. Key morphological and biochemical traits were measured three weeks after treatments. Two-way ANOVA showed significant effects of salinity and FA on growth and biochemical parameters. Under non-saline conditions, foliar FA at 0.6 mM increased root length from about 11 cm (control) to 23 cm. Even at 100 mM NaCl, FA maintained root length around 20 cm compared with 12 cm in untreated plants. Shoot fresh weight at 100 mM NaCl increased from about 13.5 g (control) to 21 g with FA (≈56% improvement). Salt stress increased malondialdehyde (MDA) to nearly 2.8 nmol g^{-1} FW, but foliar FA reduced it to ~1.5 nmol g^{-1} FW. Antioxidant enzymes also responded strongly: catalase activity under 100 mM NaCl rose from ~20 U mg^{-1} protein (control) to ~38 U mg^{-1} protein with FA. Total soluble sugars also increased with FA, particularly at 0.6 mM. These results indicate that foliar FA, especially 0.6 mM, mitigates salt-induced damage and improves growth and biochemical resilience of garlic under controlled pot conditions. This study provides novel evidence that FA can serve as a low-cost foliar amendment for enhancing salt stress tolerance in garlic.

7.14. Formulation of Biofungicide for Leaf Blight of Jasminum Sambac

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The present investigation focused on formulating an effective biofungicide from the naturally occurring phylloplane fungus of *Jasminum sambac* (Jasmine) against the leaf blight pathogen, *Alternaria alternata*. The pathogen was isolated and identified using 18S rRNA analysis. The sequence was studied and submitted to NCBI (access number PV368447). A pathogenicity test was performed to confirm the causal organism's pathogenicity. From the healthy leaves of *Jasminum sambac*, four different phyllosphere fungi, namely *Aspergillus niger*, *Cladosporium* sp., *Fusarium* sp., and *Mucor* sp., were isolated by the leaf impression

method. The colony morphology of all the isolated fungi was studied and identified by lactophenol cotton blue (LPCB) staining. An antagonist activity test was performed using the four identified phylloplane fungi against the pathogen Alternaria alternata as a screening test before the formulation of the biofungicide. *Aspergillus niger* showed the highest activity among all the fungi. Based on the antagonistic activity results, *Aspergillus niger* was selected for the formulation of a biofungicide. *Aspergillus niger* spores were separated, and the spore suspension was mixed with carrier materials (coconut oil and glycerol) at different ratios. Coconut oil-based *Aspergillus niger* biofungicide was found to be better than glycerol-based *Aspergillus niger* biofungicide. The formulation was sprayed directly onto blight-affected Jasmine leaves for 10 days. It was observed that an *Aspergillus niger* spore-based coconut oil biofungicide significantly reduced leaf spots. Advantages of this formulation include cost-effectiveness, an alternative to chemical fertilizers, eco-friendliness, and improved efficacy.

7.15. Genome-Wide Comparative Variant Analysis of MR297 Rice Variety and Its Mutant Line, ML-1, for Functional SNP/InDel Identification

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MR297 is a high-yielding commercial rice variety extensively cultivated in Peninsular Malaysia to this day, yet it lacks resistance against bacterial leaf blight disease. To mitigate this, ML-1 was derived from MR297 through acute gamma irradiation and has become a promising mutant line developed by Universiti Teknologi MARA Malaysia. During multiple field trials, ML-1 has shown improved yield potential and resistance during bacterial leaf blight outbreaks. However, comprehensive information on these genetic improvements remains limited. Here, to investigate the comparative genome-wide variations between the wild-type and the mutant line, we performed whole-genome resequencing on MR297 and ML-1 with Illumina NovaSeq™ 6000. Through bioinformatics pipelines, 97.6 and 98.4 million paired-end reads of MR297 and ML-1 were generated with sequence coverages of 39.0x and 38.8x, respectively, and were mapped to the Nipponbare reference genome (GCA_034140825.1). A total of 6,632,119 high-confidence SNPs and InDels in MR297 and ML-1 were identified and among these, 428,552 SNPs and 50,565 InDels were unique to the mutant. Variant functional annotation and effect prediction in both genotypes revealed 42,275 high-impact variants which were mapped onto 22,163 genes. Gene ontology analysis further screened for economically important genes and found 188 genes associated with disease resistance mechanisms and 333 genes associated with yield potential traits in the mutant. These data provide a basis for further analyses to validate putative variants influencing BLB disease mechanisms and may explain ML-1's improved disease tolerance. These findings serve as valuable resources for the development of allele-specific molecular markers to facilitate marker-assisted selection (MAS) and enhance the precision of future rice improvement programs.

7.16. Genotypic Response of Wheat Against Stripe Rust and Its Geospatial Distribution in Southern Punjab

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The study was conducted at the Plant Pathology Department, Bahauddin Zakariya University, Multan, during the 2023–2024 crop season. A total of 27 wheat genotypes were tested for stripe rust severity, and their correlation with environmental conditions was assessed using a Randomized Complete Block Design (RCBD) with three replications. Results showed that six genotypes were resistant to stripe rust. District-level evaluations further identified varieties with varying resistance. Several genotypes exhibited high to moderate resistance, supported by Area Under Disease Progress Curve (AUDPC) values, confirming their potential for cultivation in stripe rust-prone regions. Resistant genotypes such as Akbar-19 and Dilkash-21 displayed strong potential for breeding programs. Moderately resistant varieties like FSD-08 and Sehar-06 provided partial resistance but may require complementary disease management. In contrast, moderately susceptible varieties, including Galaxy-13 and Sadiq, highlighted the need for strict monitoring and integrated management strategies.

Disease distribution analysis across Southern Punjab revealed distinct spatial patterns. Heatmap results showed Dera Ghazi Khan, Khanewal, and Multan to be the most severely affected districts, with multiple hotspots linked to moderately susceptible cultivars and favorable environmental conditions. The choropleth map confirmed that Dera Ghazi Khan had the highest mean severity, with several fields recording AUDPC values above 287. Multan and Khanewal also faced high pressure, with many fields showing values between 289 and 293, categorized as moderately susceptible. Conversely, Vehari and Layyah recorded the lowest severity, with AUDPC values ranging from 33 to 200, classifying them as resistant to moderately resistant.

Statistical analysis revealed strong associations between disease intensity and environmental factors such as temperature, humidity, and irrigation. Districts with larger proportions of susceptible cultivars like Pak-13 and Akbar-19 showed higher rust incidence, while those planting resistant cultivars such as Dilkash-21 recorded lower severity.

7.17. Genotypic Response of Wheat Against Stripe Rust and Its Geospatial Distribution in Southern Punjab

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7.18. Harnessing the Biocontrol Potential of Syzygium cumini Essential Oil Against Ascochyta rabiei: Towards Sustainable Chickpea Production

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The increasing global population and rising food demand require sustainable agricultural approaches to enhance crop productivity while minimizing environmental impact. Chickpea (*Cicer arietinum* L.) is a vital leguminous crop and an important protein source in many developing countries; however, its production is severely affected by *Ascochyta rabiei*, the causal agent of Ascochyta blight. The present study evaluated the antifungal efficacy of *Syzygium cumini* essential oil (EO) as an eco-friendly alternative to synthetic fungicides for managing *A. rabiei* under both greenhouse and field conditions.

S. cumini leaves were collected from Bahauddin Zakariya University (BZU), Multan, Pakistan, and EO was extracted via hydrodistillation using a Clevenger apparatus. A pathogenic culture of *A. rabiei* was purified on chickpea seed meal agar, and pathogenicity was confirmed following Koch's postulates. Ten treatments, including pre- and post-foliar EO sprays, seed treatments, and fungicide controls, were arranged in a completely randomized design (CRD) in greenhouse trials and a randomized complete block design (RCBD) in field trials.

Results revealed that *S. cumini* EO significantly reduced disease severity and incidence compared to the fungicide (chlorothalonil) treatments. In greenhouse experiments, EO seed treatment and preventive foliar applications achieved up to 100% disease suppression, while fungicides showed partial control (up to 25% disease severity). Under field conditions, EO seed treatment reduced disease severity to 1.79–2.11% and disease incidence to 15–17%, with substantial improvements in plant growth and yield compared to inoculated controls. EO-treated plants exhibited longer shoots and roots, more leaves, and higher yields than fungicide-treated plants.

These findings suggest that *S. cumini* essential oil is a promising, sustainable biofungicide for effective management of Ascochyta blight in chickpea cultivation, offering an environmentally safe alternative to chemical fungicides.

7.19. Heat Stress and Transcriptome Stability: Transcriptional Defense Mechanisms in Plants

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Heat stress is one of the most critical environmental factors limiting plant growth and productivity, as it disrupts essential molecular processes that maintain cellular homeostasis. Among these, gene transcription is particularly vulnerable. During transcription, RNA Polymerase II (RNAPII) synthesizes RNA from DNA templates, but this process can be interrupted by several obstacles, such as compact chromatin structures, bound regulatory proteins, DNA lesions, or misincorporated nucleotides. These interruptions can cause

RNAPII to pause or stall, resulting in incomplete or faulty RNA transcripts that compromise gene expression and stress tolerance.

In this study, we investigated how *Arabidopsis thaliana* and barley (*Hordeum vulgare*) respond to heat-induced transcriptional stress. Using a combination of genetic, molecular, and biochemical approaches, we analysed the roles of transcription elongation factors and RNA surveillance pathways that assist RNAPII in maintaining efficient transcription under elevated temperatures. Our findings indicate that plants deficient in key elongation or transcription recovery components show reduced thermotolerance, increased transcriptional arrest, and an accumulation of aberrant RNA species.

Furthermore, comparative analysis between *Arabidopsis* and barley revealed both conserved and species-specific mechanisms that support transcriptional integrity under heat stress. This highlights an evolutionarily adaptable network that safeguards transcriptome stability in diverse plant species. Overall, our results suggest that plants depend on a coordinated system of transcriptional support proteins and post-transcriptional quality control mechanisms to sustain accurate gene expression during thermal stress. By maintaining transcriptome integrity, these processes contribute to cellular resilience and enhance the plant's overall adaptability to changing environmental conditions.

7.20. Hydromethanolic Extract of Artemisia Campestris Targets Acetylcholinesterase and Butyryl Esterase for Sustainable Insect Control

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Artemisia campestris is a medicinal plant species endemic to Algeria, particularly abundant in the southern regions and central Sahara. Its long-standing use in traditional medicine has recently gained scientific attention, prompting further investigation into its bioactive potential. This study focuses on the phytochemical composition and biological activity of its hydromethanolic extract, with particular emphasis on its ability to inhibit neural enzymes associated with insect physiology.

Preliminary screening revealed a diverse array of secondary metabolites, including tannins (catechic and gallic), flavonoids, quinones, glucosides, terpenoids, saponins, coumarins, and alkaloids; anthocyanins were not detected. Quantitative analysis confirmed high concentrations of total phenolics (80.91 $\pm$ 1.58 mg GAE/g), flavonoids (60.45 $\pm$ 2.02 mg RE/g), phenolic acids (4.24 $\pm$ 0.38 mg CAE/g), and condensed tannins (2.26 $\pm$ 0.29 mg CE/g). Enzyme inhibition assays were performed using Ellman's method, and IC_{50} values were calculated by nonlinear regression analysis based on dose–response curves. The extract demonstrated significant activity against acetylcholinesterase (AChE) and butyryl esterase (BuE), with IC_{50} values of 13.79 $\pm$ 0.79 µg/mL and 8.34 $\pm$ 0.58 µg/mL, respectively, based on five independent replicates (n = 5). These results were benchmarked against galantamine, a reference inhibitor, which exhibited IC_{50} values of 1.50 $\pm$ 0.12 µg/mL under the same conditions. Although galantamine showed superior potency, the relatively low IC_{50} values of the A. *campestris* extract highlight its promising inhibitory potential and support its relevance as a natural alternative. The findings suggest that A. *campestris* may offer a natural and environmentally friendly approach to pest control due to its ability to disrupt insect neural function. Further research is needed to validate its efficacy and safety in real-world applications.

7.21. Hydromethanolic Extract of Artemisia campestris Targets Acetylcholinesterase and Butyryl Esterase for Sustainable Insect Control

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The findings suggest that *A. campestris* may offer a natural and environmentally friendly approach to pest control, due to its ability to disrupt insect neural function. Further research is needed to validate its efficacy and safety in real-world applications.

7.22. Identification of DArT-Genomic Regions Associated with Morpho-Physiological Traits in Spring Wheat Seedlings Under Drought Conditions

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Although extensive research has been conducted on molecular breeding for drought stress tolerance in wheat, the study of drought stress at the seedling stage has not received much attention from researchers compared to other growth stages. As a result, the genetic basis of drought tolerance at the seedling stage is poorly understood, and very few genes associated with drought tolerance at this stage were identified. Therefore, there is an urgent need to concerted research efforts to explore genetic resources for drought tolerance at the seedling stage. In this study, 172 spring wheat genotypes from 20 different countries was screened under drought stress at the seedling stage. Drought stress was naturally applied by withholding irrigation for 13 days. Many morphological (leaf wilting (LW), days to wilting (DTW), days to regrowth (DTR), drought survivalrate(DSR)), and physiological traits (protein (P), amino acids (AM), proline (Pro), glucose (G) and fructose (F) were scored. The ANOVA analysis showed highly genetic differences among genotypes in all traits. The heritability (H^2) estimates were very high in all traits. The H^2 ranged from 0.84 to 0.92 for morphological traits, and from 0.97 to 0.99 for physiological traits. Moreover, significant correlations were found among traits under drought stress. Genome-wide association

analysis (GWAS) was conducted for all traits using 407 Diversity Arrays Technology (DArT) markers. The GWAS analysis at significant level of p-value ≤ 0.001 revealed 15 and one significant DArT markers associated with morphological and physiological traits, respectively. Out of all significant markers, only one marker (WPT-7063) found to be common in four morphological traits. Interestingly, four markers (TPT-3689, WPT-664309, WPT-7677, and RPT-7068) found in this study were previously reported for their association with drought tolerance in wheat. The preliminary findings of this study provided novel significant markers which, after validation, may be used in marker-assisted selection to accelerate genetic improvement of drought tolerance in wheat seedlings.

7.23. Integrating Canopy Fluxes and Biochemical Traits to Enhance Crop Resilience Under CO_2, Ozone, and Drought Stress

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Future agricultural productivity is threatened by simultaneous changes in atmospheric composition and climate, including rising CO_2, elevated tropospheric ozone, and more frequent droughts. These factors rarely occur in isolation; their interactions can strongly influence photosynthetic processes, carbon allocation, and overall crop performance. Understanding responses across scales—from cellular biochemistry to canopy-level fluxes—is essential for developing resilient crop systems capable of sustaining yields in a changing environment. **Here, we focus on maize, a C_4 crop widely grown for global food and feed production.**

In this study, we evaluated **maize** responses to combined CO_2, ozone, and drought stress under field conditions. At the leaf scale, portable gas exchange systems and biochemical assays were used to quantify Rubisco activity, electron transport rates, carbohydrate metabolism, and antioxidant enzyme function. These data were paired with measurements of stomatal behavior to assess water-use efficiency under stress. At the canopy scale, eddy covariance flux towers and remote sensing provided continuous data on carbon exchange, energy balance, and conductance across growing seasons. **Because maize uses C_4 photosynthesis, its responses to elevated CO_2 and interacting stresses provide insight into how C_4 crops may perform under future climate conditions.**

Our findings demonstrate that elevated CO_2 enhances photosynthetic carbon assimilation but does not fully compensate for ozone-induced reductions, particularly when drought intensifies stomatal closure and limits carbohydrate export. Some cultivars maintained photosynthetic capacity through strong antioxidant defenses and stable Rubisco function, while others exhibited large declines in canopy carbon gain.

These results underscore the importance of selecting and breeding crop varieties with combined biochemical, physiological, and structural resilience. By linking canopy-scale dynamics with underlying metabolic traits, this research highlights pathways for improving crop productivity and food security under the multifactorial stressors of future climates.

7.24. Microbial Interactions on Potato Cultivars 'La Strada' and 'Red Scarlet': Identifying Synergistic and Antagonistic Effects for Biological Control of Soft Rot and Blackleg

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Soft rot and blackleg, caused by Pectobacterium and Dickeya species, are destructive diseases that affect potato crops worldwide, resulting in significant economic losses. These pathogens can cause blackleg in plants and induce soft rot in tubers. Managing these diseases is challenging due to a lack of effective control methods and the high susceptibility of most potato cultivars. Biological control presents a promising alternative. However, some antagonistic microorganisms may be potential plant pathogens or interact synergistically with pathogenic bacteria or fungi. Understanding these complex interactions is crucial for developing effective strategies to enhance potato health and yield. These pathogens serve as valuable models for studying the interactions between pathogens. This study examined microbial interactions on two potato cultivars, La Strada and Red Scarlet, under controlled in vitro conditions. Potatoes were inoculated with bacterial strains *Dickeya dianthicola* (F014), *Pectobacterium brasiliense* (F126), *Pseudomonas syringae* (Ps3004), *Serratia marcescens* (Sm2018), and *Escherichia coli* (EcM14), both separately and in pairs. A 0–9 scoring system was used to assess the effects on potato health. Results showed distinct interaction patterns, identifying both beneficial (synergistic) and harmful (antagonistic) relationships. For instance, co-inoculation of F126 with either Ps3004 or Sm2018 positively influenced potato response. When F126 and Ps3004 were combined, the disease score was high at 8.00, indicating a synergistic effect. Conversely, strong antagonistic interactions were also observed. F014 displayed antagonism with Ps3004, Sm2018, and EcM14. F014 and Ps3004 pair, being the most severe, had a score of only 1.00. The study also found that potato cultivars responded differently to bacterial combinations. Red Scarlet had a disease score of 4.00 when inoculated with Sm2018 alone, while La Strada scored 0.00, indicating low susceptibility. These findings are crucial for developing targeted biological control agents, enabling the use of beneficial microbial combinations and avoiding harmful ones to enhance crop resilience and production.

7.25. Root-Trait Adaptations in Chickpea (Cicer spp.) Under Drought Stress

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Chickpea is one of the most important food legumes widely cultivated in semiarid regions. Root system architecture plays a crucial role in plant adaptation by determining soil moisture and nutrient uptake. Therefore, understanding the morphological and anatomical plasticity of root traits in chickpea is important for improving water-use efficiency and stabilising yield under water-scarce conditions. This review synthesizes literature-based findings on root-trait adaptations that contribute to drought tolerance in chickpea. The research methodology involved reviewing scientific articles from the Scopus, Web of Science, and ScienceDirect databases that were published in the last 10 years. The data, which are essential for root characteristics, root dimensions and depth, lateral root density, root-to-shoot ratio, and xylem structure, are extracted and integrated to identify drought tolerance mechanisms and physiological responses in chickpea. Multiple studies published in the last decade show that chickpea plants with deeper and more extensive root systems exhibit greater drought resistance and maintain higher yield (Chen et al., 2017; Ye et al., 2018). Plants with more roots per unit of shoot tissue, thicker roots, and longer root systems are better at reaching underground water sources. The plant's ability to maintain shoot turgor under dry soil conditions improves through anatomical changes, including larger metaxylem vessels, thicker cortical tissues, and wider vascular areas. Genetic research has identified specific quantitative trait loci (QTLs) that indicate that root structure affects drought tolerance and biomass production. Root architectural plasticity, particularly increased rooting depth and improved hydraulic anatomy, emerges as a fundamental

adaptive mechanism for drought resilience in chickpea. Modern breeding programs can develop new cultivars by assessing root traits to enhance water management and increase yields. Future research should emphasize high-throughput root phenotyping and genomic integration to uncover molecular determinants of root function under stress.

7.26. Toward Sustainable Kiwi Cultivation: Growth and Metabolic Insights from a Case Study of the Application of Amino Acid and Organic-Based Biostimulants

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The use of biostimulants is a sustainable agriculture approach as their rational application is likely to be accompanied by relying less on synthetic inputs. Investigating the application of biostimulants is considered more significant than ever, especially in the commercially important species *Actinidia deliciosa* L., in the face of climate change. In this work, the application of a glycine–betaine–proline-based biostimulant (GBP) and a humic and fulvic acid-based biostimulant (HF) was evaluated on the growth and metabolism of kiwi trees. The study was conducted at a commercial organic kiwi orchard in order to assess the effects under actual commercial field conditions. Three treatments were established: treatment GBP, treatment HF, and the control treatment C (absence of biostimulants). Throughout the two-year experiment, leaf areas (cm^2) were measured in the field, and the metabolic traits such as total phenolic content (mg GAE g^{-1} Dry Leaf), proline concentration (μmol g^{-1} Fresh Leaf), and chlorophyll content (μg cm^{-2}) were analyzed in the Productive Agriculture and Plant Health Laboratory in the Department of Agriculture of the University of Ioannina, to investigate the presence of abiotic stress among the treatments. The metabolic data showed that the kiwi trees of the GBP treatment were more robust, as indicated by the chlorophyll and proline analysis. This vigor of GBP treatment was also represented in leaf area when compared to the C. The implementation of biostimulants constitutes an ecological approach that can be integrated into biological crop management, as it is environmentally friendly, non-invasive to the ecosystem, and aims to promote crop resilience to biotic or abiotic stress.

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