Congresso congiunto UZI-SIP 2026

85° Congresso dell'Unione Zoologica Italiana

35° Congresso della Società Italiana di Protistologia

Milano, 8 -11 Settembre 2026



RIASSUNTI

Unione Zoologica Italiana

UNIVERSITAS STUDIORUM MEDIOLANENSIS

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

SOCIETÀ ITALIANA DI PROTISTOLOGIA ETS

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Benvenuti a Milano!

Sinossi programma

	Martedì 8	Mercoledì 9		Giovedì 10		Venerdì 11	
		MAIN	parallela	MAIN	parallela	MAIN	parallela
09:00		Plenary		Plenary		Plenary	
09:30							
10:00		SIMP 2	SIMP 3	SIMP 5	WS	SIMP 6	SIMP 7
10:30							
11:00		Coffee		Coffee		Coffee	
11:30		SIMP 2	SIMP 3	SIMP 5	WS	SIMP 6	SIMP 5
12:00							
12:30							
13:00		Lunch break		Lunch break	Lunch break	Lunch break	
13:30							
14:00					WS		
14:30	Saluti	SIMP 2	SIMP 4	SIMP 5		Selected posters	
15:00	Plenary						
15:30		SIMP 3			SIMP 6	Chiusura	
16:00	Coffee						
16:30	SIMP 1	Coffee		Coffee			
17:00		Poster session	Tavola Rotonda	Assemblea UZI	Assemblea SIP		
17:30							
18:00							
	Welcome cocktail				Poster session WS		
18:30							
19:00							
19:30							
20:00				Cena			

CODICE SIMPOSIO	TITOLO
SIMPOSIO 1	Evo-Devo: dalla morfogenesi all'epigenomica
SIMPOSIO 2	Zoologia al confine: multi, inter, trans-disciplinarità
SIMPOSIO 3	Protistologia
SIMPOSIO 4	Adattamenti fenotipici ed interazioni: dalla cellula al comportamento
SIMPOSIO 5	Impatti ambientali e strategie di conservazione della biodiversità
SIMPOSIO 6	Evoluzione molecolare, filogenesi e tassonomia
SIMPOSIO 7	Divulgazione, didattica e museologia
Tavola Rotonda	Digitalizzazione e barcoding della biodiversità italiana: where now, what' s next?
WS	Workshop - Modelli invertebrati: una reale opportunità nell'applicazione del principio delle 3R

RELAZIONI
SU INVITO



Martedì 8 settembre, ore 15.00, Aula Magna, via Festa del Perdono 7

ALESSANDRO MINELLI

Department of Biology, University of Padova, Italy

Building an animal, deconstructing the rules

With the advent of evolutionary developmental biology, the emergence of new living forms is no longer considered solely as the result of adaptive success. There are even cases of saltatory evolution that seem to give substance to Goldschmidt's idea of hopeful monsters: a hitherto unrecognized example is provided by the forelimbs of the male of the dolichopodid fly *Campsicnemus magius* (Loew) that precisely match the morphology of schistomelic teratological appendages. The evo-devo perspective allows us to explain a number of unusual and even extreme phenotypes in parasitic or commensal animals. Their postembryonic development can be influenced by the host, even beyond a recognizable adaptive value, whereas the free-living embryonic and larval stages are unaffected. A striking example is provided by the often dramatically asymmetric bopyrid isopods. Asymmetry is otherwise absent in isopods, but is present and often conspicuous in decapods, of which bopyrids are parasites. The symmetry or asymmetry of the host seems indeed to dictate the symmetry or asymmetry of the parasite, beyond any visible adaptive explanation, and is apparently reversible: the bopyrid *Pseudione tuberculata*, which lives on *Paralomis granulata*, is practically symmetrical, like the "carcinized" anomuran that hosts it. A second example is offered by the ascothoracid crustaceans of the genus *Dendrogaster*, whose nauplius transforms into a starlike adult that appears to be drawn on the body cavities of the hosting starfish. Finally, in some copepods parasitic of fish such as *Lernaea* and in some staphylinid beetles and phorid flies associated with termites, the body shape of the parasite or inquiline changes enormously in the postimaginal phase following the first contact with the host. Case by case, it will be worth researching how far these changes in the late phases of development are still dependent on environmental cues provided by the host, how much instead have been internalized with changes at the genome and gene level.

Mercoledì 9 settembre, ore 9.00, Salone Pio XII di Casa Schuster, via S. Antonio 5

CARLA LANGELLA

DiARC Department of Architecture, University of Naples Federico II

Biomimetic design as an interdisciplinary practice

In the history of design, nature has always been an important reference, both formally and functionally, as it can guide the development of efficient, effective and original products. Today, within the international scientific design community, terms such as bionics, bionic design, biodesign, biomimicry, biomimetics, biomimetic design, biologically inspired design and bio-inspired design define different design approaches based on the transfer of knowledge, principles, logics, functions and structures derived from biology into product and service innovation processes, with the aim of making them more sustainable, adaptable, efficient and appealing. Biomimetic design is based on the analogy between design issues and biological needs: a principle of abstraction that makes it possible to translate biological features into artificial or hybrid solutions. This contribution aims to provide an overview of biomimetic design, exploring its characteristics from a cultural, methodological and critical perspective, in order to highlight its potential as well as its limits, ambiguities, risks and grey areas. More specifically, the talk will examine the importance of including zoological expertise in the biomimetic design process, preferably through the direct involvement of zoologists and the acquisition of scientific data and knowledge. Zoology represents a particularly relevant field for biomimetic design, as animals often display highly complex functions, dynamic behaviours and adaptive models when compared to other types of organisms. Their movement strategies, sensory systems, structural solutions, interaction patterns and responses to environmental constraints offer rich references for the development of innovative, efficient and scientifically grounded design solutions. This is why the contribution of zoological knowledge is essential to practising biomimetics in a rigorous and meaningful way. The contribution will present the design experiments and research activities carried out within the Hybrid Design Lab, a laboratory of the DiARC dedicated to the mutual relationships between design and biosciences, with particular attention to the activities conducted in collaboration with Prof. Sugni's research group at the University of Milan. Semantic choices, market impact, methodological effectiveness, interdisciplinary collaboration and ethical and environmental consequences are among the issues that will be analysed. These themes have been selected by considering open and unresolved questions within the international state of the art, with the aim of outlining possible evolutionary strategies for the biomimetic approach and relating it to the current social, economic, political and technological scenario.

Mercoledì 9 settembre, ore 10.00, Sala Armida Barelli di Casa Schuster, via S. Antonio 5

NAOMI A. STOVER[1], MARCUS J. WATSON[2], JUNHUA NIU[2,3], SHAN GAO[2,3]

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TetraEpiHub: a community resource for *Tetrahymena thermophila* epigenetic research

Molecular biology research routinely produces crucial large-scale genomic, transcriptomic, and epigenomic datasets. However, the value of these data depends on whether they can be explored, interpreted, and reused by the broader research community. Ciliate.org was developed to support ciliate research by providing community genome annotation databases and other online tools for studies of *Tetrahymena* and other ciliates. As a companion site to Ciliate.org, we recently created TetraEpiHub, a new resource designed to make *Tetrahymena thermophila* epigenomic and transcriptomic datasets more accessible to researchers. TetraEpiHub highlights data used in the latest *T. thermophila* gene model reannotation, including N6-methyladenine (6mA), H3K4me3, histone variant distributions, and transcriptome evidence. These datasets contributed to the most up-to-date gene models available for the community and remain an important resource for studying epigenetics, genome architecture, and gene regulation in this species. TetraEpiHub also provides aggregated and gene-by-gene summaries of epigenetic features, along with community epigenetic data from mutant strains, protocols, and ordering information. By integrating annotation, visualization, and practical community resources, TetraEpiHub supports reuse of complex genomic datasets and provides a platform for exploring epigenetic regulation in *Tetrahymena*. TetraEpiHub is available at tetraepihub.ciliate.org.

Giovedì 10 settembre, ore 9.00, Salone Pio XII di Casa Schuster, via S. Antonio 5

MICHELA PACIFICI

Dipartimento di Biologia e Biotecnologie Charles Darwin, Università di Roma “La Sapienza”, Viale dell’Università 32, Rome 00185, Italy

Global assessment of hunting risk for terrestrial mammals

Persistent unregulated and illegal hunting has led to pervasive defaunation, especially in the tropics, including reductions in population sizes, loss of large-bodied species, and alteration of community structure, even in landscapes that remain forested and ostensibly undisturbed. Mammals are among the taxa most affected by hunting, with 'hunting and trapping of terrestrial animals' listed as a threat for 692 species classified as threatened on the IUCN Red List. Yet, despite its recognised importance as a threat, the ecological and spatial dynamics of illegal hunting remain poorly understood relative to other drivers of biodiversity loss. Where illegal hunting occurs, it is shaped by a complex interplay of environmental and anthropogenic factors, as well as by intrinsic characteristics of species that can make them a target for specific types of hunting (e.g., large herbivores are commonly targeted as a food source). I will describe the main results of two projects aimed at identifying the intrinsic traits affecting the vulnerability of terrestrial mammal species to hunting for food, and present the first spatially explicit assessment of illegal hunting risk, irrespective of the reasons for hunting. These works provide the first quantitative baseline of illegal hunting pressure for terrestrial mammals, offering a tool to support conservation prioritization, protected area planning, and the strategic allocation of enforcement efforts in terrestrial mammal conservation.

Venerdì 11 settembre, ore 9.00, Salone Pio XII di Casa Schuster, via S. Antonio 5

TELMO PIEVANI

Dipartimento di Biologia, Università di Padova, Viale G. Colombo 3 – Via U. Bassi 58/B, Padova

Philosophy of evolution

What is humanity's place in the great Darwinian Tree of Life? What laws drive evolution? How do species arise? What makes biology unique as a science? How did typically human consciousness and language evolve? Over the past three decades, life sciences have seen an extraordinary acceleration of knowledge and new techniques. Evolutionary biology today raises philosophical and ethical questions, profoundly altering our ideas about nature. Many debates, often superficially addressed in the mass media, concern natural history, genetics, human evolution, and progresses in Darwinian explanations. In short, there is a great deal of philosophy when we talk about evolution, between progress and catastrophe, gradualism and punctuationism, nature and culture, form and function, chance and necessity. Furthermore, many discoveries updated, integrated and revised the evolutionary research program: epigenetics; symbioses; horizontal gene transfer; plasticity; structural constraints; group selection; the interactions between biological and cultural evolution. Many species (humans above all) actively modify their environment and must then adapt to a world they themselves have transformed: this becomes an evolutionary and philosophical key to deal with the current environmental crisis. The philosophy of evolution can be an effective guide for addressing the challenges of the present with a broad perspective across space and time, ranging from pandemics (another evolutionary and ecological process rarely presented as such to the general audience) to the biodiversity crisis, from our coevolution with technology to anthropic global warming. Evolution is the transformation of the possible, and our evolution has only just begun.

Presentazioni orali

Simposio 1

EVO-DEVO: DALLA MORFOGENESI ALL'EPIGENOMICA



FILIPPO NICOLINI[1], DAENIA LA RODÉ[1], ALESSANDRA ALEOTTI[2], JULIEN DEVILLIERS[3], CHRISTOPHER KAY[4,5], DAVIDE PISANI[4,5], ROBERTO FEUDA[6]

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[2] Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
[3] Biochemistry Department, Virginia Tech, Blacksburg, Virginia, USA
[4] School of Biological Sciences, University of Bristol, Bristol, UK
[5] School of Earth Sciences, University of Bristol, Bristol, UK
[6] Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy

Tracing the evolutionary origin of the animal phototransduction toolkit through comparative genomics and single-cell transcriptomics

Light detection is a fundamental process in animals, and its evolutionary history has been puzzling biologists for a long time: Darwin himself called the vertebrate camerated eyes as “organs of extreme perfection and complication”. More than 150 years later Darwin’s reasoning, while comparative studies have clarified the evolution and diversity of animal photoreceptors, the emergence of the underlying molecular toolkit remains difficult to reconstruct. Here, we investigate the evolutionary history of the phototransduction (PT) machinery across early-diverging metazoans, by integrating broad scale comparative genomics and single-cell transcriptomics, to understand how new specialized cell types can arise and diversify. This integrative approach enabled us to reconstruct the early evolutionary history of key PT components and the underlying gene regulatory networks. Despite the remarkable anatomical and physiological diversity of non-bilaterian animals, we found that the core molecular toolkit of PT was already present in the Metazoa common ancestor. Here, extensive gene duplication occurred and drove the establishment of the PT machine through the progressive assembly and diversification of its molecular components, which produced a conserved yet flexible system.

To then gain insights into the interplay between genome and cell evolution, we generated a single-cell atlas of the ctenophore *Pleurobrachia pileus*, which belongs to the most ancient animal phylum with a full PT machinery. The dataset is the second single-cell atlas for the phylum and comes 8 years after the first was published (the atlas of *Mnemiopsis leidyi*), thus representing a fundamental advancement. After integrating the newly generated atlas into a wide phylogenetic context spanning Cnidaria, Placozoa and major Bilateria, we were able to assess the degree of molecular homology between different PT cells, reconstructing their transcriptional identity across phylogeny. We found that in non-bilaterian clades, and specifically in ctenophores, the regulatory toolkit of PT cells is quite diverging and do not overlap with those found in bilaterians, yet several transcription factors appear to be recurrently transcribed. Altogether, these findings point towards a complex evolutionary history, where key components of the PT cascade are consistently used across PT cells, but are then regulated and rewired in a clade-specific manner, mirroring the great diversity of extant structures.

FEDERICA MONTESANTO

Fondazione IMC (International Marine Center), Via Domenico Millelire, 09170 Torre Grande, Oristano, Italy

Microbial biodiversity and developmental plasticity in marine invertebrate model systems

Animal development is increasingly understood as the outcome of interactions between host genetic programs, environmental signals, and associated microbial communities. In marine invertebrates, these interactions may be particularly important during morphogenesis, life-cycle transitions, and the establishment of symbiosis. Cnidarians offer powerful systems to investigate how microbial diversity contributes to developmental plasticity and host resilience. This study uses cnidarian model systems to explore how bacterial and fungal communities vary across symbiotic states and developmental stages. Using eDNA metabarcoding, host-associated microbial assemblages were characterized during symbiont acquisition and under different algal-associated microbial contexts, including natural, microbiome-depleted, and physiologically impaired symbiont treatments.

Microbial community profiles were integrated with observations of key developmental and morphogenetic processes, including asexual propagation, tissue remodeling, and life-cycle progression. The results indicate that microbial communities are dynamic during development and symbiosis establishment, and that microbial context can influence the timing and success of developmental transitions. These findings support the view that cnidarian development is shaped not only by the host and its algal symbionts, but also by broader microbial interactions within the holobiont.

By combining invertebrate model systems with eDNA metabarcoding, this study links microbial biodiversity to morphogenesis, symbiosis, and developmental plasticity. More broadly, it highlights the potential of molecular biodiversity tools to reveal hidden biological interactions that influence organismal development and ecosystem-relevant processes.

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1 Department of Life and Environmental Sciences –Polytechnic University of Marche, Ancona (Italy)
2 National Biodiversity Future Center (NBFC), Palermo (Italy)
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4 CoNISMa, Roma (Italy)

Unusual mating: description of a new reproductive mode in the Mediterranean gorgonian *Leptogorgia sarmentosa* (Esper, 1791)

Mediterranean gorgonians are gonochoristic exhibiting either internal or external fertilization. In the latter case, gorgonians may be broadcast spawners or surface brooders. Although the reproductive strategies of Mediterranean corals are generally well understood, here we illustrate an undescribed mating strategy and larval developmental stages observed in *Leptogorgia sarmentosa* (Esper, 1791), an octocoral widely distributed in the basin, growing on rocky, detritic and even artificial substrates. This species is known to be a broadcast spawner, with female and male colonies releasing oocytes and spermatic cysts, respectively, into the water column at the end of summer (August-September). In this reproductive mode, the released spermatic cysts are expected to rupture to free the sperms that, being able to swim, can reach and fertilize the oocytes.

Sixteen specimens of *L. sarmentosa* recovered from lost fishing gears were maintained in aquaria until gamete release. Then, oocytes and sperm cysts were collected and kept in multiwell plates to observe mating and larval development. Through histology and both optical and scanning electron microscopy, we provide data on morphology and size of the germinal cells, with oocytes being 330 ± 0.06 µm in size (MIN= 180 µm; MAX= 430 µm) and spermatic cysts 244 ± 0.10 µm (MIN= 80 µm; MAX= 510 µm). *In vitro,* we also detected multiple times and described for the first time the unexpected fusion between a spermatic cyst and an oocyte prior the fertilization. These observations suggest that gametogenesis is likely synchronous in males and females and that this mechanism enhances the chance to mate of relatively shallow-water octocorals. Moreover, here we present the first-ever description of the early phases of the embryogenesis up to the formation of the planula larvae, demonstrating that new reproductive traits and peculiarities continue to be discovered even in well studied cnidarians. In conclusion, we cannot rule out the possibility that other species may also exhibit reproductive modes not yet described.

LUCAS A. PROCOPIO VICTOR[1], FILIPPO NICOLINI[2], GIOVANNI PICCININI[1], FABRIZIO GHISELLI[1], LILIANA MILANI[1]

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2 Division of Genetics and Genome Biology, School of Biological and Biomedical Sciences, University of Leicester, Leicester, United Kingdom

Ubiquitin-dependent control and selection of mitochondria in gonads and early embryos of the Mediterranean mussel *Mytilus galloprovincialis*

In almost all animals, mitochondria are exclusively inherited from the mother, which helps avoiding detrimental effects associated with heterogeneous mitochondrial population. The only known evolutionarily stable exception is the doubly uniparental inheritance (DUI) found so far in 100+ bivalve species. Such a system generates naturally heteroplasmic individuals with two divergent mitogenomes with specific distribution patterns depending on the sex of the embryo. This feature could significantly enhance the study of mitochondrial transmission both during cell divisions and from parents to progeny. Nonetheless, a lack of knowledge on the molecular mechanisms controlling mitochondrial inheritance in bivalves hinders the adoption of bivalves as a model system, a gap that persists across eukaryotes generally.

Based on previous results of *in silico* analyses on DUI bivalves, in this study we investigated the potential role of the Ubiquitin-Proteasome pathway (UPS) in mitochondrial inheritance, a process already documented in mammals. We investigated UPS role during gametogenesis by immunolocalizing Ubiquitin and mitochondria in the DUI species *Mytilus galloprovincialis*; then, in early embryos, while tracing sperm-derived mitochondria, we immunolocalize UPS factors together with Prohibitin (a mitochondrial protein ubiquitinated in mammal sperm).

Our results confirm that mitochondria in spawned sperm are ubiquitinated and may undergo a Ubiquitin-dependent degradation during the first cleavage in female embryos. In most eggs and early embryos, a strong concentration of Ubiquitin and Prohibitin is localized at the two poles, one including the polar bodies at the animal hemisphere and the other at the vegetal hemisphere, where maternal factors addressed to the D lineage localize. While the polar Ubiquitin may be linked to actin cytoskeleton organization regulating pole plasm inheritance among blastomeres, the polar Prohibitin may represent mitochondria either addressed for elimination in the polar bodies, as suggested for mammals, or actively pumped into the blastomeres inheriting the pole plasm (D lineage). This would imply a departure from the passive inheritance of mitochondria during embryo early cleavage typically assumed in early animal cleavage.

Overall, our study strongly supports DUI bivalves as a model group for studying mitochondrial transmission and highlights new potential areas of study for mitochondrial biology.

FILIPPO NICOLINI[1,2], MARIANGELA IANNELLO[1], FEDERICA SALATIELLO[3], GIOVANNI PICCININI[1], MIRKO MARTINI[1], SERGEY NUZHDIN[4], ANDREA LUCHETTI[1], FABRIZIO GHISELLI[1], ENRICO D'ANIELLO[3], LILIANA MILANI[1]

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Embryonic development and germline specification in bivalves: insights from single-cell and transcriptomic analyses

A central question in developmental and evolutionary biology is how germline specification and sex determination are established during early ontogeny. Bivalves represent an intriguing model due to their remarkable diversity in reproductive systems, yet their early embryonic mechanisms remain poorly understood. This study investigates the molecular signatures of early development in the Mediterranean mussel (*Mytilus galloprovincialis*) utilizing an integrative approach that combines single-cell RNA sequencing (scRNA-seq), bulk transcriptomics, and hybridization chain reaction (HCR).

We took advantage from the possibility of identifying sex in early embryos of bivalves with doubly uniparental inheritance (DUI) of mitochondria, according to mitochondrial distribution patterns at the very first embryonic cleavages. We applied scRNA-seq up to the blastula stage in the DUI species *M. galloprovincialis*. We analysed clustering of cells, coexpression networks, and GO terms to formulate hypotheses about cell identity. The majority of coexpression networks was more transcribed in male cells than in female cells, but no strong signal supported the recognition of a specific germline cluster, and no known sex determination gene was transcribed.

To validate our scRNA-seq analysis, we used HCR to detect the germline marker Vasa up to the D-veliger larva. We confirmed that Vasa does not show any gradient across blastomeres during *M. galloprovincialis* early embryogenesis. This sharply contrasts with the maternal asymmetric deposition (preformation) reported in *Crassostrea gigas*, revealing a substantial diversity in germline programs of bivalves. We jointly assessed the transcription patterns of three genes associated with sex determination in bivalves, namely *Fox-L2*, *Dmrt-1L*, and *Sox*-H. Only *Fox-L2* was transcriptionally active from gastrulation onwards, but without sex-specific patterns. The identified transcription patterns were also validated by re-analysing a developmental time series RNA-sequencing dataset.

These data challenge the assumption of a conserved early developmental program in bivalves, suggesting that germline specification may be quite diverse in terms of time and mode. Our observations also indicate that sex determination potentially relies on other, not yet identified, genes and that sex specific differentiation can start much later in development, which potentially explains plasticity and occurrence of sex change during bivalve adulthood.

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Evolution of reproductive strategies in ascidians: insights into the development of *Clavelina lepadiformis*

Ascidians are sessile tunicates that exhibit a remarkable diversity of reproductive and developmental strategies. In particular, ovoviviparity and viviparity evolved independently on multiple occasions, in parallel with the emergence of coloniality, whereas solitary ascidians are oviparous. These strategies are exemplified by the solitary phlebobranch *Ciona robusta* and the colonial stolidobranch *Botryllus schlosseri*, well-established model organisms in developmental biology. *C. robusta* releases large numbers of small eggs into seawater, where external fertilization occurs. In contrast, *B. schlosseri* alternates sexual and asexual reproduction through budding. This ovoviviparous species produces only a few eggs and embryos are brooded within the parental zooid. During development, the oviduct differentiates into a placental cup that provides structural support to the embryos without supplying nutrients.

To investigate the evolution of developmental strategies in colonial ascidians, we characterized the life cycle of the aplousobranch *Clavelina lepadiformis*, a common colonial species in the Mediterranean Sea. In this species, zooids are connected at their bases by stolons while remaining otherwise anatomically distinct. Embryos are brooded within the atrial cavity, and fully developed larvae are released into the surrounding seawater. Embryonic development was analyzed using *in vivo* observations and confocal microscopy, whereas the anatomical relationships between brooded embryos and parental tissues were examined by transmission electron microscopy.

The developmental timeline of *C. lepadiformis* reveals a prolonged embryogenesis associated with the formation of presumptive adult organs before larval release, consistent with a developmental strategy that has convergently evolved among colonial ascidians. Ultrastructural analyses further confirmed the absence of maternal nutritional provisioning during embryogenesis. These findings support the hypothesis that brooding evolved repeatedly in colonial ascidians, irrespective of their phylogenetic position, while maintaining embryonic support without direct maternal nutrient transfer.

CHIARA ANSELMI[1], TOM LEVY[2,3], JIAMU C. YU[2], KATHERINE J. ISHIZUKA[2,3], KARLA J. PALMERI[2,3], DANIEL LIU[2], RAHUL SINHA[2], YOTAM VOSKOBOYNIK[4], MARIALIA COCO[1], CLELIA CORRIDORI[1], NORMA F NEFF[5], IRVING L. WEISSMAN[2,3,6], GRAZIANO MARTELLO[1]*, AYELET VOSKOBOYNIK[2,3]*

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Redeployment of developmental programs during adult neural renewal in the colonial tunicate *Botryllus schlosseri*

The colonial tunicate *Botryllus schlosseri* provides a unique evo-devo model to investigate how developmental programs are redeployed during adult tissue renewal. During its weekly blastogenic cycle, each adult zooid undergoes degeneration and is replaced by a new generation developing within the same colonial system. In this process, multiple organs, including the neural complex, are rebuilt at each cycle, offering an unusual opportunity to study how changes in developmental timing, tissue patterning, and cellular plasticity contribute to nervous system remodeling in an invertebrate chordate. Here, we combined Smart-seq3 single-cell transcriptomics, spatial gene expression analysis, proliferation assays, and cell-tracking experiments to define the cellular and molecular organization of the *B. schlosseri* neural complex. Clustering identified six transcriptionally distinct cell states, revealing that the adult neural complex is a heterogeneous and dynamic tissue composed of neuronal, progenitor-like, epithelial-like, mesenchymal-like, and transitional populations. Marker analysis identified neuronal and sensory-associated genes, including *NALCN*, *UNC79*, *UNC80*, *CDH23*, and cholinergic pathway components, together with regulatory and progenitor-associated genes such as *SOX2*, *MSI1*, *HES1*, *NOTCH*, and *NEUROG1*. These signatures suggest the coexistence of differentiated neural identities with progenitor-like, neuroepithelial, and early neural commitment programs. Spatial validation by HCR RNA-FISH confirmed distinct expression domains within the adult brain, neural-gland-associated territories, surrounding mantle tissues, and developing buds. Proliferation and cell-tracking experiments further suggest that neural-complex-associated cell states are embedded within broader programs of adult tissue morphogenesis and blastogenic remodeling. Together, these data provide the first single-cell view of the *Botryllus* adult neural complex and reveal a cellular landscape in which mature neural identities coexist with immature, neuroepithelial, and remodeling-associated states. This work establishes *B. schlosseri* as a powerful comparative chordate model to investigate how conserved developmental gene regulatory programs are reused, spatially reorganized, and integrated with adult regeneration and non-pathological degeneration during evolution.

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Comparing chromatin accessibility profiles in two marine animals, the Japanese clam and the European sea bass

Genetic variants in non-coding regulatory elements are supposed to explain a large fraction of phenotypic variation within and across species. Identification of such non coding regulatory elements, however, remains more difficult than annotating coding regions. Moreover, the functionality of regulatory elements is generally tissue-type specific, which requires the application of such approaches in different tissues. The assay for transposase-accessible chromatin using sequencing (ATAC-seq) provides an effective way to detect the unique chromatin landscape associated with a cell/tissue type. Patterns of chromatin accessibility are thought to be connected with the cell-specific gene expression patterns that mould identity and functions of cells and tissues.

Here we report chromatin accessibility atlases for two marine species, the Japanese clam *Ruditapes philippinarum,* and the European seabass *Dicentrarchus labrax.* Using ATAC-seq on four tissue samples from five individuals of *R. philippinarum* and for four tissues from six individuals of *D. labrax,* an average of 103’000 open chromatin regions per sample were identified in Japanese clam and 66’000 ones in European sea bass. A first significant difference between the two species was the proportion of peaks falling into three categories of genomic regions: putative promoters (15% peaks in sea bass, 12% in clam), gene bodies (respectively 64% and 30%), and intergenic regions (21% vs 58%). After peak import, unsupervised Principal Component Analysis on each consensus peakset revealed that samples were clustered by tissue in the case of European seabass, while they clustered by individuals in the case of Japanese clam. Discriminant Analysis (DA) with permutations was successfully used to significantly discriminate the four tissues, identifying tissue-discriminant peaks for both species. Only in *R. philippinarum* DA significantly discriminate between individuals. Individual-discriminant were significantly over-represented in intergenic regions, therefore in putative distal enhancer elements. While enhancers have been shown to evolve rapidly, the inter-individual variability observed here is rather extreme. It might represent a substantial source of intra-specific variation in gene regulation that might translate into phenotypic variation across individuals.

Analysis of ChIP-seq and RNA-seq data on the same samples are currently ongoing, to further elucidate the observed phenomenon at different levels of epigenetic regulation.

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Conserved endocrine actors orchestrate maturation and reproductive timing in annelids and vertebrates

Reproduction is a cornerstone of life, yet its underlying mechanisms have been continuously reshaped by evolution, yielding an extraordinary diversity of organismal strategies. Given this vast phenotypic diversity, extensive genomic reshuffling, and lineage-specific gene dynamics, it was long assumed that distinct, non-overlapping molecular machineries orchestrated reproduction across major bilaterian lineages.

Building on our previous identification of a *gonadotropin-releasing hormone* (*gnrh*)-like gene that modulates reproductive timing, we here provide evidence for a conserved neuroendocrine architecture in the marine annelid *Platynereis dumerilii*. More recently, RNA-seq analyses on delayed-maturation mutants identified a variety of differentially expressed genes typically associated with vertebrate reproduction. Interestingly, several of these genes were lost in ecdysozoans, causing them to be historically mischaracterized as vertebrate innovations. Among the most consistently regulated candidates are orthologs of vertebrate nuclear receptors, such as *dax-1* (*nr0b1/2*) and *sf-1* (*nr5a1/2*), and steroidogenic enzymes (e.g., *dhr11*, *cyp17a1*, and *srd5a1*). Notably, multiple candidates exhibited expression fluctuations tied to the lunar cycle, which synchronizes spawning in this and many other marine species. By generating functional mutants for *Platynereis nr0b1/2*, we investigated its phenotypic roles, revealing significant impacts on fertility, maturation kinetics, and somatic growth. Molecularly, *nr0b1/2* appears to coordinate a complex network encompassing orthologs of steroidogenic enzymes (e.g., *dhr11*, *cyp17a1*) and nuclear receptors (including *sf-1* and estrogen-like receptors) known to regulate vertebrate gametogenesis and maturation. Finally, in line with observed shifts in deiodinase expression, pharmacological assays demonstrate a key role for the conserved thyroid hormone pathway in gating growth, maturation, and reproductive timing.

Although our analyses also highlight taxon-specific differences, our study uncovers striking similarities in the molecular toolkits driving sexual maturation in annelids and vertebrates. These findings indicate that these core endocrine mechanisms were likely already present in the urbilaterian ancestor, carrying profound evolutionary and ecological implications.

Simposio 2

ZOOLOGIA AL CONFINE: MULTI, INTER, TRANS-DISCIPLINARITÀ



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The first whole genome sequencing of *Zu cristatus* (Bonelli, 1819) using hybrid technology

Teleost genomics is affected by costs and difficulties that have led to few whole genomes being fully sequenced and well-assembled (mainly model or commercial species). These limitations cause a scarcity of data on zoologically interesting taxa with no commercial value. Among these, Lampriformes order species arouse interest concerning their adaptations to deep-sea environments. Indeed, these species, such as the Trachipteridae *Zu cristatus*, pass from shallow waters in juvenile stages to deep oceans in adult life, hiding adaptive mechanisms still largely unknown. Nowadays, through innovative technologies, it is possible to sequence entire genomes quickly, cheaply, and accurately.

Here we report the first *Z. cristatus* whole-genome sequencing procedures and results using Illumina and Nanopore technologies, obtaining a hybrid assembly. An adult sample from the Ionian Sea was processed at the University of Messina, Italy; total genomic DNA was extracted from 0.25 mg of epaxial muscle via a mechanical glass-bead disruption method following the standard protocol suggested by Qiagen for the Blood and Tissue Kit. After quality analyses, the DNA was purified and sent to Galseq for the preparation of two short-read libraries (300 and 550 bp), sequenced in paired-end mode (2×150 bp) on an Illumina HiSeq 4000 platform. The second DNA sequencing approach, pointing to long reads, was carried out with Nanopore technology at Nord University of Bodø, Norway. To increase DNA quality, the Zymoclean Large Fragment DNA Recovery Kit was used following the isolation. After integrity was assessed using the DNA broad-range tape on the TapeStation System 4150 (Agilent), the library was prepared using the Ligation Sequencing Kit (Oxford Nanopore) and sequenced on MinION Mk1c using a flow cell FLO-MIN106D. Illumina, Nanopore, and hybrid assemblies registered 179,698, 77,328, and 19,153 contigs, respectively; only the hybrid draft showed low fragmentation (82% completeness) compared to genome sizes of 751,753,356 bp, 626,095,177 bp, and 750,136,548 bp, allowing further downstream investigations. This result confirms the validity of using hybrid reads to achieve a better final output, compared to the use of only short-read libraries in the assembly of large teleost genomes.

These results respond to the necessity of deepening molecular database resources, enhancing knowledge of rare fish species through next-generation sequencing.

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The perception of chemical and mechanical stimuli during blood feeding of the yellow fever mosquito *Aedes aegypti*

The yellow fever mosquito *Aedes aegypti* must feed on human blood to acquire the nutrients needed for egg development and reproduction. After locating a host and landing on human skin, female mosquitoes start biting this tissue in search of a blood vessel, in a process called intradermal probing. Female mosquitoes have mouthparts specialized to pierce, including a pointy stylet called labrum. Within the mouthparts, the labrum forms the food canal and is the only stylet bearing sensory neurons. The dendrites of labral sensory neurons reach four pairs of sensilla located at the tip of this organ. The labrum sensory neurons can perceive chemical stimuli which could guide the movement of the mouthparts to a blood vessel during intradermal probing. We are interested in the role of different labrum sensory neurons in sensing chemical and mechanical stimuli during blood feeding.

To investigate this topic, we developed a microfluidic chamber which can be used to provide different chemical and mechanical stimuli to the mosquito labrum. To test this system, we have performed preliminary experiments using the GMO mosquito strain brunchpilot (brp)>jGCaMP7s-T2A-tdTomato, which allows to check the activation of neurons expressing the calcium sensor jGCaMP7s under the control of the pan-neuronal marker brp after providing a given stimulus in *ex vivo* experiments. In the future, we plan to perform similar experiments on labral neuronal populations specialized in sensing chemical or mechanical stimuli by generating GMO strains driving the expression of jGCaMP7s under the control of population-specific gene markers. To assess which gene markers could be representative of these labral neuronal populations, we selected putative candidate genes based on the *Aedes aegypti* Mosquito Cell Atlas and checked their expression in the labrum of mature female mosquitoes by reverse transcription (RT)-PCR. The putative gene markers of the mechanosensory neuronal population were selected to perform an additional RT-PCR on the labrum of female mosquitoes at different developmental stages, i.e., newly emerged, 5-7 days old and 12-13 days old mosquitoes, and *in situ hybridization* experiments.

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Transforming waste into protein-based materials: a multidisciplinary perspective on *Hermetia illucens* larvae bioconversion

The treatment and valorization of food waste represent major environmental and economic challenges worldwide. Approximately 1.3 billion tons of the organic fraction of municipal solid waste (OFMSW) are generated annually, highlighting the urgent need for sustainable waste management strategies. Among the most promising approaches, insect-based bioconversion has emerged as an effective solution for transforming organic waste into high-value biomolecules, including proteins and lipids, suitable for a wide range of industrial applications. In this context, the saprophagous larvae of the black soldier fly (*Hermetia illucens*, BSFL) have attracted considerable attention because of their exceptional ability to efficient convert organic substrates into valuable biomass. This biological process offers a sustainable alternative for reducing OFMSW while generating environmentally friendly products that support circular bioeconomy principles.

The present study evaluated the growth performance of BSFL reared on OFMSW, their bioconversion efficiency, and the potential exploitation of insect-derived proteins for bioplastic production. Bioconversion performance was assessed through waste reduction and conversion efficiency parameters, confirming the ability of BSFL to effectively valorize the OFMSW. Interestingly, larvae and pupae obtained at the end of the bioconversion process exhibited comparable protein content. Protein extracts from both developmental stages were used for producing biodegradable protein-based films. The resulting biofilms showed good thermal stability and overall structural integrity, although their mechanical properties, particularly flexibility and strength, differed according to the protein source. In particular, films prepared from pupal protein extracts exhibited significantly higher strain and stress at break than those obtained from larval protein extracts. Furthermore, life cycle assessment and socioeconomic analysis were performed to evaluate the environmental and scalability of the proposed process and to identify critical stages for impact reduction.

Overall, this multidisciplinary study demonstrates the feasibility of integrating BSFL-mediated OFMSW bioconversion with the production of insect-derived bioplastics, providing a valuable, sustainable, and circular strategy for organic waste valorization. The study was funded by Fondazione Cariplo (2020-0900).

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The ecological role of substrate-borne vibrations in insects and plants

Substrate-borne vibrations represent a widespread and effective channel of information transfer in terrestrial ecosystems. Unlike airborne signals, substrate-borne waves propagate through solid media such as soil, plant tissues, and other substrates, providing reliable information over short to medium distances. In recent years, growing evidence has highlighted the ecological importance of these signals across insects and plants. They mediate a variety of intraspecific interactions, including mate finding, alarm signalling, and the coordination of social behaviours, as well as interspecific interactions such as prey and host detection and predator avoidance. In insects, substrate-borne vibrations play a key role in host location, particularly for species whose life cycles depend on finding concealed hosts. Vibrational cues generated by host movement or feeding activity can be exploited by predators and parasitoids to improve host-location efficiency.

Beyond animal communication, plants can also perceive and respond to vibrational stimuli in their environment. Recent studies have demonstrated that vibrations associated with phytophagous insect feeding can induce defensive responses, including the activation of chemical defences in the form of airborne volatile organic compounds (VOCs) and antifeedant compounds. These findings suggest that vibrational cues may function as an early warning system in plant-insect interactions.

Here, we present two case studies on parasitoid insects belonging to Bethylidae and Pteromalidae (Hymenoptera) which use vibrational cues to locate concealed hosts, and a case study showing how feeding vibrations produced by *Eurydema oleracea* (Hemiptera: Pentatomidae) trigger the emission of defensive VOCs in *Arabidopsis thaliana* (Brassicaceae) plants. Together, these case studies reveal the broad ecological significance of substrate-borne vibrations across different kingdoms of life. Understanding how vibrational signals mediate host location in parasitoids and defence induction in plants provides new insights into the complexity of ecological interactions and may contribute to the development of innovative strategies for sustainable pest management and crop protection.

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Overlooked soil engineers: digger bees induce small-scale alterations of soil chemical properties and microbiome

Healthy soils provide habitats for biodiversity, contribute to climate resilience and stable food systems. However, current land-use change is altering soils and eroding natural landscapes. Consequently, several European initiatives have emerged to protect soils and identify new relevant indicators of soil health. While several animal taxa are well known as positive soil bioturbators, wild bees remain largely overlooked as soil-modifying agents, even though over 70% of wild bee species nest in the ground.

Here, we address this knowledge gap by focusing on the chemical and microbiological interactions between ground-nesting bees and the soils they inhabit, in line with the EU Soil and Biodiversity Strategy for 2030. We collected topsoil samples from nest entrances of the ground-nesting bee *Lasioglossum malachurum* and paired control soils (non-nested topsoil), together with bee specimens, across four sites within an agricultural area of a protected park in central Italy. Following previous findings in ants, well known as soil engineers, we hypothesised that: 1) nest soils would show a shift in pH towards neutral values; 2) nest soils would be richer in organic matter and ions, and display a lower C:N ratio, compared with control soils; and 3) the taxonomic overlap between bee and nest soil microbiome (i.e. bacteria and fungi) would be greater than that between bee and control soil microbiotas. Ground-nesting activity was associated with localised changes in soil properties, with nest soils showing significantly pH closer to neutral values and reduced C:N ratios than control soils. No significant differences emerged in the overall bacterial and fungal community structure between nest and control soils, suggesting the absence of wide microbiome restructuring. However, bee microbiome shared more OTUs with nest soils than with control soils, indicating a selective microbial interface between the host and its nesting environment.

Overall, these findings suggest that ground-nesting bees may act as small-scale soil modifiers, influencing soil chemical properties and microbial interactions without substantially altering the bulk soil community. These findings highlight the need to include ground-nesting bees in soil conservation frameworks, coupling pollination and soil biodiversity management.

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Shared history, divergent paths: lineage-specific drivers of macroevolutionary diversification in dragonflies (Odonata, Anisoptera)

Dragonflies are one of the oldest extant lineages of winged insects and have persisted through profound climatic shifts and structural ecosystem transitions over their evolutionary history. The currently most-supported models – based on molecular phylogenies of about 500 species – emphasise transitions between lentic and lotic environments as drivers of diversification rate changes. However, only very few of the factors potentially affecting evolutionary rates have been studied to date in dragonflies.

Here, we investigate the diversification of this clade in a vigorous manner, using a novel phylogenetic hypothesis and explicitly testing the factors affecting rates of speciation and extinction. Specifically, we inferred a novel time-calibrated molecular phylogeny with 1,019 species, which is the most comprehensive species-level phylogeny for this group to date. We then compared in a maximum-likelihood framework a series of time-dependent and paleoenvironmental-dependent models, including models with rate shifts in *a priori* identified clades. These analyses partially supported previously identified diversification shifts associated with transitions between lentic and lotic environments but also revealed that such transitions alone are inadequate to fully explain the complex diversification of Anisoptera. We, therefore, turned to models which infer diversification shifts along the tree and tested for several ecological factors, observed in extant species, as predictors of tip speciation rates via phylogenetic generalized least squares (PGLS). We found several bioclimatic, ecological, and morphological predictors that significantly affect tip speciation rates in this group. Most importantly, the extent to which these predictors affect speciation (and sometimes the sign of the effect) greatly varies across anisopteran families.

In conclusion, our results demonstrate that a single, simple explanation is inadequate to describe the evolutionary history of such a major, old, insect clade. Indeed, the macroevolutionary success of Anisoptera across geological time likely reflects a mosaic of family-level responses to climate and habitat structure, rather than a single shared factor affecting diversification.

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Insect wings as multifunctional surfaces

Insect wings are not only important for flight. Their cuticular surface, characterised by micro- and nanostructures, has evolved a variety of additional functions that range from the manipulation of light to the control of wettability. These functions place wings at the interface between zoology, biophysics and materials science, and contribute to fundamental ecological processes such as mate recognition, intraspecific communication and predator avoidance. Cuticular micro- and nanostructures have been described across a wide range of insect orders, where they are known to contribute to structural colouration, transparency, antireflection and water repellency.

Here, we present four case studies on insects from different orders that illustrate this functional diversity. In the mayfly *Ecdyonurus* gr. *venosus* (Ephemeroptera), transparent wings reflect UV light through an angle-dependent multilayer mechanism, with female wings reflecting significantly more UV than males, a difference that is consistent with a visual cue during mating. In the moth *Eriogaster catax* (Lepidoptera), the white wing spot shared by both sexes acts as a broadband structural reflector, reflecting about 80% of incident light in the visible and 30–40% in the UV. In the lacewing *Chrysoperla carnea* (Neuroptera), preliminary data on the transparent wings shows a marked UV reflectance together with a reduced reflectance in the visible, whose structural origin and ecological role are currently under investigation. Finally, in the invasive weevil *Aclees taiwanensis* (Coleoptera), epicuticular waxes deposited on the elytra produce a superhydrophobic behaviour, with wax deposition modulated by ambient humidity.

Taken together, these case studies illustrate how the comparative study of wing surfaces can clarify ecological functions in different insect orders and, at the same time, provide useful models for the design of bioinspired surfaces.

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Subterranean fragmentation in the Italian Classical Karst underlies cryptic lineage distribution in the olm (*Proteus anguinus*)

Subterranean ecosystems are characterized by strong spatial fragmentation and limited connectivity, promoting genetic divergence in specialized cave organisms. The olm (*Proteus anguinus*), Europe's only troglobitic vertebrate, is an ideal model to investigate how subterranean hydrology influences evolutionary differentiation.

Here, we analysed the distribution, genetic structure, and body condition of olm populations in the Italian Classical Karst, the species' westernmost natural range. We conducted the largest systematic survey of Italian olms to date, sampling 136 individuals from nine hypogean sites along the Reka-Timavo River system and the Soča-Isonzo aquifer. Genetic variation was assessed using the mitochondrial cytochrome c oxidase subunit I (COI) marker. Lineage distribution showed a clear spatial pattern linked to the hydrological network. The Ljubljanica lineage predominated at the upstream Trebiciano Cave (65%), whereas 1 km downstream, at Luftloch Cave, the Karst lineage became dominant (73.3%), remaining the only lineage detected further downstream and within the Soča-Isonzo aquifer. This abrupt transition suggests that hydrological discontinuities restrict connectivity and maintain lineage segregation. Genetic analyses indicate that the deviation from neutrality reflects the coexistence of distinct evolutionary units rather than demographic decline. Morphometric analyses revealed significant differences in allometric patterns among populations. Within the Reka-Timavo system, Karst and Ljubljanica lineages displayed contrasting growth trajectories, suggesting lineage-specific ecological strategies, whereas intra-lineage differences were mainly associated with environmental variation.

Overall, the Reka-Timavo system acts as a structured evolutionary corridor where lineage identity, habitat conditions, and resource availability shape population differentiation. The upstream sector, where both lineages are sympatric, represents a conservation priority due to its high genetic and phenotypic diversity. These findings highlight the need to integrate genomic, ecological, and hydrogeological approaches to conserve biodiversity in fragmented subterranean ecosystems.

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The AlienFish project: integrating citizen science and molecular tools to monitor fish diversity in the Mediterranean sea

Monitoring fish diversity in the Mediterranean is becoming increasingly challenging due to the joint effects of biological invasions, climate-driven range shifts, and the occurrence of poorly known species. Traditional survey approaches are often insufficient to obtain timely and geographically extensive information, both due to the limited number of observers and to unresolved taxonomic issues. This highlights the need for complementary monitoring approaches capable of involving a broad network of observers, while improving taxonomic knowledge of neglected taxa.

Within the AlienFish project, we leverage citizen science through social networks to collect observations of non-native, rare, and otherwise relevant species in the Mediterranean. To support identification efforts and clarify the taxonomic status of some poorly known taxa, molecular analyses are also being conducted on selected taxa. Among these, complete mitochondrial genomes are being reconstructed for medusafishes (Centrolophidae), a historically overlooked family whose taxonomy remains poorly resolved.

Between 2020 and 2025, the project collected and validated 317 records – including over 70 fish taxa and more than 700 individuals – along Italian coasts. Native Osteichthyes represented the largest component of the dataset, followed by Chondrichthyes, non-native Osteichthyes, and neonative Osteichthyes. Starting from 2025 the dataset was further expanded through the integration of records collected within the framework of “Attenti a quei 4!”, a national awareness and monitoring campaign promoted by ISPRA and the Italian National Research Council. Records were provided by both amateur observers and professional fishermen. Among non-native fishes, *Siganus luridus*, *Fistularia commersonii*, and *Lagocephalus sceleratus* represented 88.1% of the records, suggesting their continued expansion in Italian waters. The dataset also included deep-sea, cryptobenthic, and conservation-relevant species. Our results demonstrate the potential of citizen science to generate high-quality biodiversity data across broad spatial and temporal scales. Integrating these observations with targeted molecular approaches further enhances species identification and contributes to resolving long-standing taxonomic ambiguities in poorly studied fish groups. In this context, newly generated complete mitochondrial genomes provide reference data for taxa that were previously inadequately represented in public molecular databases.

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Mating behaviour, sexual selection and sensory cues involved in partner detection and recognition in the black soldier fly*:* a multidisciplinary approach

The black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae), is a key species for industrial waste management and bioconversion. However, adult biology remains poorly understood, and reproduction is still one of the least explored aspects of BSF biology.

Using an interdisciplinary approach combining behavioural, morphological, electrophysiological, and chemical analyses, we are investigating mating behaviour, sexual selection, and the sensory cues involved in sex recognition under controlled conditions. Observations of male–female and male–male interactions showed that: (1) flight behaviour triggers males to grasp females and force them to the ground, possibly because the blue wing reflection acts as a visual stimulus promoting mating attempts; (2) males that spend more time grasping other males have a higher probability of mating successfully; (3) larger females with a greater number of mature eggs are more likely to mate; and (4) mated females complete egg maturation faster than virgins. These findings support the hypotheses of male mate choice and nuptial gifts in BSF. The coexistence of male-male competition and male preference for highly fecund females is consistent with a partial sex-role reversal. In contrast, our results do not support the occurrence of lekking behaviour.

To investigate the sensory basis of adult behaviour after emergence, we are characterising the cuticular hydrocarbons (CHCs) profiles of males and females under different physiological conditions (just emerged and mated). Moreover, we found that antennal sensilla of both sexes respond similarly to volatile organic compounds emitted by decaying substrates and these odours elicit sex- and stage-specific behaviours: males are consistently repelled, whereas females are repelled immediately after emergence but become attracted after reaching sexual maturity and requiring oviposition sites. These results suggest that olfactory cues guide the movements of *H. illucens* adults among emergence, mating, and oviposition habitats.

Further studies on sexual selection and mate recognition in both wild and captive populations are needed to improve mass-rearing practices. Confinement in industrial colonies likely disrupts natural behaviours, and a deeper understanding of BSF behaviour and physiology in both settings will help maintain genetically diverse captive populations and design rearing environments that better promote insect welfare.

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Sea urchins at the border: intersections between zoology and design

Biomimicry is a highly transdisciplinary research field that integrates Life and Applied Sciences (e.g. architecture, design, and engineering) to translate biological principles into innovative technological solutions. A key prerequisite for this process is a thorough understanding of the morpho-functional adaptations underlying the evolution of biological structures. In this context, sea urchins represent an outstanding source of bioinspiration because of their remarkable adaptive specializations.

This study focuses on two skeletal elements of *Paracentrotus lividus* -pedicellariae and the madreporite- as models for an integrated biomimetic approach. Their morphology and function were investigated using stereomicroscopy, confocal microscopy, scanning electron microscopy (SEM), and video recordings. Subsequently, zoologists and designers collaborated through a hybrid biomimetic workflow to identify and translate key biological principles into bioinspired design concepts using an analogy-of-function approach. Comparative analyses of the different pedicellarial types revealed both shared structural features, including tripartite gripping, multilevel valve hinges, and rod-like skeletal trabeculae, as well morphotype-specific adaptations reflecting their distinct biological functions. These principles inspired the development of several design concepts, including an innovative litter-plogging stick. Likewise, the madreporite was confirmed to consist of parallel channels lined by a densely ciliated epithelium that regulates seawater inflow into the water vascular system while acting as a highly selective filter against micro- and nanoparticles. These fluid-transport mechanisms inspired the design of a bioinspired fruit bowl.

Overall, this study expands current knowledge of the anatomy, morphology, and functional organization of *P. lividus* pedicellariae and the madreporite. Moreover, it demonstrates how the integration of zoological research and product design can successfully translate biological principles into innovative design solutions, highlighting the potential of sea urchin morphology -and zoology more broadly- as a valuable source of technological innovation.

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The PALLIPES project: integrating eDNA and field monitoring for transboundary white-clawed crayfish conservation plans

The endemic freshwater crayfish *Austropotamobius pallipes* is a key sentinel species for assessing the ecological status of freshwater ecosystems. Its populations are increasingly threatened by habitat degradation, competition with invasive crayfish, and infection by *Aphanomyces astaci*, the causative agent of crayfish plague, highlighting the need for coordinated transboundary conservation. Within the Interreg VI-A Italy-Slovenia PALLIPES project, a joint monitoring campaign was conducted to assess the distribution of *A. pallipes* and the occurrence of its main biological threats, providing the scientific basis for coordinated conservation and management across the Italian-Slovenian border.

An extensive environmental DNA (eDNA) survey was carried out during the 2025 vegetative season to detect *A. pallipes*, *Procambarus clarkii*, and *A. astaci*. To validate molecular results, conventional trapping combined with individual marking was performed at selected sites.

The eDNA assay showed high diagnostic performance for *A. pallipes*, successfully detecting most populations confirmed by trapping while maintaining satisfactory discrimination between occupied and unoccupied sites. Sites positive for eDNA but negative through trapping may reflect low-density populations, limited capture efficiency, temporal mismatches between sampling methods, or downstream transport of eDNA rather than true false-positive detections.

Detection performance improved during summer, likely owing to increased biological activity and greater DNA release into the environment. eDNA detection also increased with trapping abundance classes. Although eDNA is not a direct proxy for population abundance, molecular detection became consistently reliable in sites supporting relatively well-established populations, suggesting the existence of a biological detection threshold.

Comparison between molecular and conventional surveys provided a robust framework for evaluating methodological performance and the diagnostic reliability of the eDNA approach. Overall, the cross-validation confirms that eDNA is a highly sensitive tool for mapping *A. pallipes* populations while simultaneously identifying areas exposed to invasive crayfish and crayfish plague. Although conventional trapping remains essential for detailed population assessment, integrating molecular and traditional methods provides a reliable framework for transboundary monitoring and conservation management.

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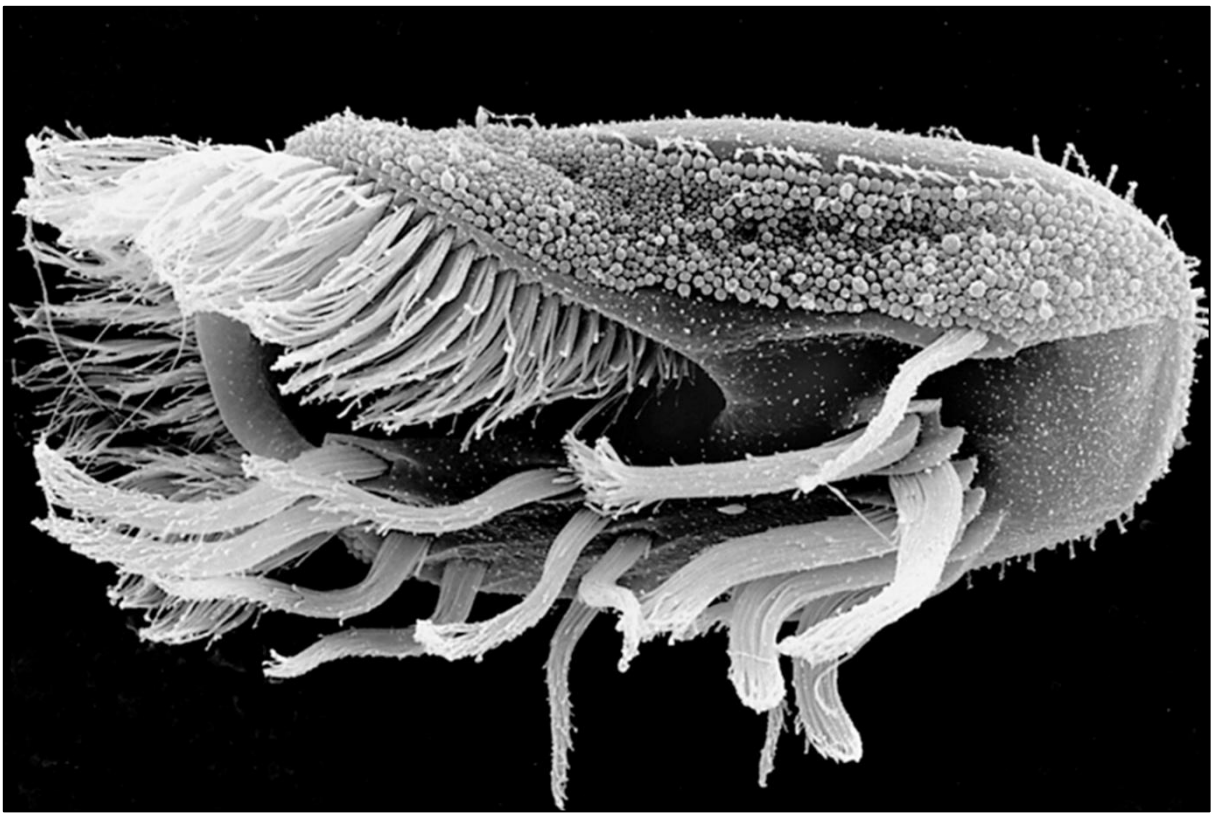
Empusiellaceae, a novel family of endosymbiotic *Holosporineae* (*Alphaproteobacteria*), have adapted to microoxic environments of termite and ruminant guts through horizontal gene transfer

UBA3830 is a poorly investigated alphaproteobacterial lineage, known exclusively from gut metagenomes of termites and vertebrates, particularly ruminants. Here we present a novel member of this clade, "*Candidatus* Empusiella dogieliae", and characterized it as an intracellular symbiont of *Foania dogieli*, a gut flagellate protist from the lower termite *Kalotermes flavicollis*. Through an in-depth phylogenomic approach, accounting for sequence composition biases, we established that UBA3830 represent a novel family, which here we name "*Candidatus* Empusiellaceae", affiliated to the suborder *Holosporineae* (*Rhodospirillales*), a lineage of bacterial endosymbionts of diverse protists. Genomic analyses revealed that "Candidatus Empusiellaceae" are widely distributed in ruminants, chicken, termites and other arthropods, suggesting an endosymbiotic condition for the entire family. Although most major genus-level clades are specifically associated to a single animal host group, their overall phylogeny is largely inconsistent with their hosts, indicating repeated host switches.

The predicted metabolic and functional properties of "*Candidatus* Empusiellaceae" are consistent with an obligate host-associated condition, typical among *Holosporineae*, particularly the high reduction of biosynthetic and energy production pathways, and the presence of multiple secretion systems, which may modulate host interactions. Moreover, distinctive genes, such as those for anaerobic pyruvate oxidation and isoprenoid biosynthesis, were likely acquired through various horizontal gene transfer events from other bacteria. These acquisitions most likely enabled the fine-tuned adaptation to anoxic or microoxic environments, possibly representing the basis for the widespread distribution of "*Candidatus* Empusiellaceae" in intestinal habitats.

Simposio 3

PROTISTOLOGIA



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Bacterial symbionts of Ethiopian *Euplotes eurystomus*: discovery of a novel gammaproteobacterial genus

Euplotes eurystomus is a freshwater ciliate whose African populations and associated bacterial symbionts remain poorly investigated. Here, we provide an integrative redescription of *E. eurystomus* from alkaline lakes in Ethiopia and characterize its intracellular bacterial consortium, with particular emphasis on a newly discovered gammaproteobacterial symbiont. Morphological and molecular evidence confirmed the assignment of the Ethiopian material to *E. eurystomus* and its placement within Clade B of *Euplotes*, a lineage frequently associated with bacterial endosymbionts. Symbiont screening revealed the presence of three bacterial lineages: *Polynucleobacter necessarius*, "*Candidatus* Cyrtobacter zanobii", and a previously undescribed bacterium, here proposed as "*Candidatus* Tedlabacter euplotophilus" gen. nov., sp. nov. The latter symbiont represents the most remarkable finding of this study. It was localized in the cytoplasm of *E. eurystomus* by a species-specific FISH probe and showed a low 16S rRNA gene identity to its closest described relatives, "*Ca.* Ovatusbacter abovo" and "*Ca.* Fiscibacter pecunius". Phylogenetic and phylogenomic reconstructions placed this novel taxon within the order "*Candidatus* Azoamicales", in a strongly supported clade together with "*Ca.* Fiscibacter" and "*Ca.* Ovatusbacter". Genome-resolved analyses allowed the recovery, assembly and annotation of the complete genome of "*Ca.* Tedlabacter euplotophilus", which represents the first complete annotated genome available for its clade. Overall, the newly generated phylogenomic evidence supported a systematic revision of the order "*Ca.* Azoamicales".

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Unveiling novel bacterial symbionts from historical laboratory strains of *Euplotes crassus*: genomic analysis and phylogenomics

Ciliates evolved in complex symbiotic associations with a broad diversity of bacterial lineages, and the genus *Euplotes* represents one of the most well-documented models within the framework of symbiotic evolution. In particular, *Euplotes crassus* is an established model due to its cosmopolitan distribution and high genetic plasticity, which likely reflects an equally diverse and mostly unexplored microbiome. In this study, we characterized the bacterial symbionts associated with historical laboratory strains of *E. crassus* from the Prof. Dini collection (University of Pisa). These strains, originally isolated from different geographic regions and in different historical moments, have been maintained under stable laboratory conditions for over 40 years, providing a controlled context to investigate long-term symbiotic persistence. Following Whole Genome Amplification of monoclonal cells, genomic data were obtained via Illumina sequencing. The bioinformatic pipeline included *de novo* assembly with SPAdes and the Blobology method to discriminate symbiont-derived contigs from the host genome based on GC content, reads coverage and preliminary taxonomical annotation of assembled sequences. To reconstruct evolutionary background of identified bacteria, phylogenomic analysis was performed using Maximum Likelihood approach. Our results indicate that these symbionts belong to distinct bacterial clades, including representatives of a novel family of Opitutae (Verrucomicrobiota*)*, as well as already known symbionts *Francisella adeliensis* and "*Ca.* Bandiella numerosa" (Pseudomonadota*)*. This highlights how symbiosis is maintained even within long-term laboratory lineages and the importance of modern genomics tools in uncovering hidden microbial diversity associated with ciliates, providing data for the taxonomic and evolutionary reconstruction of poorly studied symbiotic bacterial lineages.

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Insights into temperature adaptation in *Euplotes* species: membrane fluidity and regulatory genes

Ciliated protozoa are cosmopolitan eukaryotic microorganisms. Among them, species of the genus *Euplotes* are well adapted to aquatic and marine ecosystems and play a crucial role in the microbial loop, which is essential for aquatic food webs. Some species, such as *E. crassus* and *E. focardii*, have been extensively characterized using omics technologies, making them ideal models for investigating cellular and organismal responses to environmental changes and pollutant exposure. In the Antarctic species *E. focardii*, we analyzed the cellular response to increasing concentrations of the plastic-derived contaminant bisphenol A (BPA) using an integrated molecular approach based on membrane fluidity measurements and differential gene expression analysis. Membrane fluidity, assessed by fluorescence anisotropy, decreased markedly following BPA exposure, consistent with BPA-induced membrane stiffening. Membrane fluidity also decreased with increasing temperature, suggesting that this Antarctic species may be particularly vulnerable to the combined effects of pollutant exposure and climate change. In parallel, anisotropy measurements in *E. crassus* strains, highly tolerant of temperature fluctuations, revealed a greater capacity to modulate membrane fluidity, suggesting that membrane fluidity may serve as an indicator of temperature adaptation. In the present study, we also focused on an equatorial African *Euplotes* strain identified as *E. vannus*, a sibling species of *E. crassus*, according to the protocol described by Di Giuseppe et al. (2022) and confirmed by 18S rDNA gene sequencing. Italian strains of this species, collected from the Tyrrhenian Sea, were found to be highly sensitive to changes in temperature and salinity, whereas the African strain exhibited an even narrower tolerance range. Based on the hypothesis that membrane fluidity regulation represents a primary response to temperature adaptation, we measured membrane anisotropy in the African *E. vannus* strain at different temperatures and compared the results with those obtained for *E. crassus* strains. We also investigated the expression of conserved desaturase genes potentially involved in membrane fluidity regulation. In parallel, we performed genomic analysis of the African *E. vannus* strain using Oxford Nanopore sequencing and are currently conducting the annotation to compare these sibling species.

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The intertidal ciliates *Euplotes euryhalinus* and *E. charon* recycle and use the carotenoid pigment, canthaxanthin, for shielding their cell body against chemo-physical environmental stresses

One of the six or seven clades that are commonly recognized in the phylogenetic tree of one of the most speciose ciliate, *Euplotes*, includes species living in intertidal and estuarine pools. Constantly exposed to largely fluctuating variations in salinity, solar irradiation, temperature and oxygen concentration, these habitats impose the adaptive evolution of highly specialized and hardy lifestyles on their inhabitants. Working on cultures of *E. euryhalinus* and *E. charon* populations, the former sampled from rocky shore pools along the coasts of Baia Terra Nova (Ross Sea, Antarctica) and the latter from estuarine pools along the Mediterranean coasts, it was observed that periods of intense feeding on the green alga *Dunaliella* systematically resulted in starving cultures exceptionally characterized by a pink/orange color. At the origin of this color there is an impressive cytoplasmic accumulation of a pigment that has been isolated and structurally characterized as canthaxanthin. This keto-carotenoid, commonly synthesized from bacteria, algae and fungi, is well known to act as a potent antioxidant and anti-inflammatory molecule, and its presence within *E. euryhalinus* and *E. charon* cells well accounts for their exceptional resilience to face strong oxidative and UV stresses and produce extracts with high antioxidant activity. No enzyme specific for an autonomous canthaxanthin biosynthesis has been identified in *E. euryhalinus* and *E. charon* transcriptomes, implying that these species acquire this pigment from their largely preferred diet on green algae. While most *Euplotes* species appear capable of degrading carotene derivatives (their starving cultures are regularly whitish), *E. euryhalinus* and *E. charon* may have lost this capacity and invested in this loss to gain an adaptive advantage to thrive in the challenging environment of intertidal and estuarine pools.

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Salinity stress adaptation in the Antarctic ciliate *Euplotes focardii*: roles of the bacterial consortium and secondary metabolites

In extreme environments, symbiotic interactions are often critical for host adaptation and survival, providing physiological and metabolic advantages under stress. *Euplotes focardii*, an Antarctic marine ciliate, hosts a complex bacterial consortium whose role in stress response remains underexplored. This study investigated the dynamics of this microbial community under environmental stress by RT-qPCR, using primers specific to five isolated bacterial strains to track their fluctuations in the host. We also examined the growth, cell division, and transcriptional responses of *E. focardii* under salinity stress and exposure to secondary metabolites produced by its Antarctic bacterial consortium—specifically carotenoids, pyoverdine, and pyocyanin. For comparison, the same analyses were conducted on the mesophilic congeneric species *E. crassus*. Both *E. focardii* and *E. crassus* were cultured under optimal laboratory conditions and exposed to carotenoids (10–60 µg/mL), pyocyanin (100 mg, 10–60 µg/mL), and pyoverdine (10–60 µg/mL). Five biological replicates were used for each species, with samples collected and counted at 6, 12, and 24 hours, then daily until 15 days of exposure. The total RNA was extracted at different salinities, reverse-transcribed into cDNA, and analyzed via qPCR targeting stress-related genes (HSP70) and telomerase, with the 18S rRNA gene as the housekeeping control. Relative gene expression was statistically evaluated using two-way ANOVA. This analysis revealed that salinity fluctuation changes the consortium abundance and stimulates the expression of Hsp70 and telomerase genes. Physiological profiling revealed that carotenoids consistently promote growth and protection across all salinity levels. In contrast, pyocyanin significantly inhibited cell division, particularly at higher salinity. Notably, the Antarctic *E. focardii* exhibited greater sensitivity to the metabolites and extreme salinities compared to *E. crassus*.

These findings underscore the complexity of bacteria-protist-chemical interactions and position polar ciliates as valuable bioindicators for climate-driven ecosystem variation.

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A taste for ciliates: compared predatory behavior by *Lacrymaria venatrix* with different preys

Lacrymaria is a genus of ciliates known for predatory behavior and for frequently having highly contractile necks used in hunting. While the type species *L. olor* is frequently object of study for its predation-related biomechanics, its prey choice is largely unexplored. We performed preliminary microcosm assays to evaluate the interaction between the recently described *L. venatrix* and different ciliate preys. *Paramecium aurelia*, used for regular maintenance of *L. venatrix* cultures, was tested as reference and compared both with prey-free conditions and with six alternative preys, namely *P. bursaria*, *P.* cfr*. calkinsi*, *P. nephridiatum*, *P. polycaryum*, *P. caudatum*, and *Euplotes* sp. We recorded the presence of surviving and dead but not ingested preys, as well as abundance and morphological/behavioural state of predators, in order to estimate the predatory performance and feeding behaviour of *Lacrymaria*. Of the species considered, *P. aurelia* supported well predator survival and growth; *P. nephridiatum* showed the strongest (indirect) evidence of consumption, though *P. caudatum* supported the highest predator growth; *P. polycaryum* showed complete reduction of lived prey but lower inferred consumption, indicating that prey killing does not necessarily lead to feeding; *P. calkinsi* showed the lowest inferred consumption and negative predator growth, revealing unsuitable as prey; *Euplotes* sp. was also reduced and somewhat consumed, showing that *L. venatrix* is not limited to preys of genus *Paramecium*. Correlation analyses show no significant association between prey size and inferred consumption, nor predator growth. Overall, these results suggest that *L. venatrix* can feed on a broader prey range than expected, depending on prey morphology and defenses, ingestion efficiency, and nutrient density.

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A mosquito-associated ciliate with a taxonomic problem: the case of *Lambornella*

The ciliate genus *Lambornella* (family Tetrahymenidae) was originally described by Keilin in 1921 and includes taxa capable of alternating between free-living and mosquito-associated stages. The genus, due to the facultative parasitic lifestyle of some species and the presence of free-living lineages, represents a promising model system for studying the evolution of parasitism within *Tetrahymenidae*. Despite its biological interest, the genus lacks a modern comprehensive taxonomic approach and available genomic resources. The taxonomic history of *Lambornella* is complex and has involved multiple revisions, reflecting its close relationship with *Tetrahymena* and the challenges of delimiting these genera. Although *Lambornella* was established as a distinct genus based on morphological characteristics, its phylogenetic affinity with *Tetrahymena* highlights the need for an assessment combining morphological and molecular evidence. Nevertheless, a formal modern revision integrating morphological characterization and molecular genetic data is still lacking. Moreover, the absence of available material representing the type species, *L. stegomyiae*, further complicates the modern taxonomic assessment of the genus. To stabilize the taxonomic status of *Lambornella*, we applied a comprehensive morphological and molecular approach. We assessed morphological, taxonomically relevant features of *Lambornella* and conducted phylogenetic analyses based on nuclear SSU (18S) rRNA and mitochondrial COI sequences from a broad sampling of *L. clarki* isolates collected along the Pacific Coast (Washington–Oregon–California). In addition, we assembled a draft genome of *L. clarki*, obtained from macronucleus-enriched DNA. Whole-genome sequencing was performed using a combination of short-read (NovaSeq 6000) and long-read (Oxford Nanopore Technologies) sequencing technologies. The complete linear mitochondrial genome, approximately 48 kb in length, was also extracted from the genome assembly. Together, these data provide a thorough approach for stabilizing the current taxonomic status of *Lambornella*. The draft genome represents the first genomic resource for this facultative parasite and provides a basis for future comparative and evolutionary studies.

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Epixenosomes as evolutionary innovations in defensive symbiosis: bacterial cell biology repurposed for host protection

Unicellular eukaryotes, such as ciliates, frequently establish intimate symbiotic associations with bacteria, providing powerful models to investigate the evolutionary origins of complex host–microbe interactions. A striking example is represented by *Euplotidium* ciliates, which harbour epixenosomes: defensive bacterial epibionts belonging to the *Planctomycetota–Verrucomicrobiota–Chlamydiota* superphylum. Epixenosomes protect their host from predatory ciliates through a remarkable suicidal mechanism based on the rapid extension of an extrusive apparatus (EA). Although this defensive function has long been known, the cellular architecture, molecular basis and evolutionary origin of this system remain poorly understood. Here, we investigate epixenosomes as an example of bacterial innovation associated with defensive symbiosis, combining multi-scale imaging with genomic and proteomic analyses. Cryo-electron tomography of environmental samples, together with genomic data, suggests that the EA is a refractile-body-like proteinaceous ribbon whose extension leads to lysis of the epixenosome cell. We further show that the EA is associated with contractile injection systems (CISs) encoded in the epixenosome genome, suggesting that this defensive mechanism may rely on the coordinated action of distinct bacterial structures that have been evolutionarily integrated into a host-protective function. Intriguingly, the epixenosome genome also encodes several cytoskeletal eukaryotic signature proteins, including tubulins, actin and profilin, which are highly expressed. Epixenosome tubulins (Etubs) form polymers that localise around the EA, indicating that bacteria-associated cytoskeletal elements may contribute to the organization or deployment of the defensive apparatus. We propose that, once triggered, the EA ejection in an Etub-associated manner causes epixenosome lysis and release of CISs into the environment. Free CISs may then target predators, thereby protecting the ciliate host. Overall, epixenosomes represent an exceptional case of defensive symbiosis in which bacterial cell biology, host protection and evolutionary innovation converge, offering new insight into how complex molecular machines can be repurposed during the evolution of intimate symbiotic associations.

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Design and biological characterization of a novel Climacostol-Inspired antioxidant small molecule

Climacostol is a natural resorcinolic lipid produced by the freshwater ciliate *Climacostomum virens*, displaying antimicrobial, cytotoxic, and anticancer activities. To extend its biological potential, we designed and synthesized the novel compound (Z)-4-(5-(3,5-dihydroxyphenyl)-2-hydroxypent-3-en-1-yl)benzene-1,2-diol, a small molecule inspired by both climacostol and quercetin, with the aim of combining the biological properties of these two natural compounds. The biological activity of the newly synthesized molecule was assessed in different freshwater ciliate species and in human THP-1 cells. Unlike native climacostol, the compound showed no detectable toxicity toward the ciliate species tested. Interestingly, in selected ciliates it significantly enhanced cell proliferation by increasing the division rate. Moreover, the molecule exhibited marked antioxidant activity, protecting ciliates from hydrogen peroxide-induced oxidative stress at concentrations corresponding to the LC_{50} of H_2O_2. The antioxidant potential was also confirmed in differentiated human THP-1 cells, where the compound was not cytotoxic, and significantly reduced H_2O_2-induced cell damage, restoring cell viability after oxidative damage. These preliminary findings demonstrate that rational structural modification of the climacostol scaffold can deeply alter its biological profile, generating a novel non-toxic molecule with both antioxidant and pro-proliferative properties. This compound represents a promising lead for the development of new bioactive molecules targeting oxidative stress.

Simposio 4

ADATTAMENTI FENOTIPICI ED INTERAZIONI: DALLA CELLULA AL COMPORTAMENTO



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Decoding thermal stress memory in the manila clam *Ruditapes philipparum*: from phenotypic to transcript and epigenetic reprogramming

Marine heatwaves are possibly the most immediate threat to coastal benthic organisms, with consequences that extend beyond mortality and include impaired reproduction, altered behaviour, metabolic exhaustion, and dysbiosis of host-associated microbiome. Previous work on the Manila clam *(Ruditapes philippinarum* A. Adams & Reeve, 1850*)* showed that prolonged marine heatwaves can deeply affect organismal fitness, while inducing thermal stress memory (priming) mitigates their lethal effects. However, the molecular basis of this protective mechanism remains largely unexplored.

Here, we investigated such mechanism in *R. philippinarum* by integrating analyses at the phenotypic, transcriptomic, and epigenomic level. Clams were exposed to a non-lethal thermal priming stimulus (30˚C for 7 days), followed by 15-days recovery and then experimentally challenged with a potentially lethal marine heatwave (with temperatures oscillating daily from 31 to 35 ˚ C for 15 days). In addition to survival and physiological endpoints, we generated RNA-seq, ATAC-seq and ChIP-seq data at key time points: immediately after priming and during the early phase of the marine heatwave challenge. This design allowed us to capture both the establishment of the primed state and its functional deployment during the onset of acute thermal stress.

Preliminary multi-omic evidence suggests that thermal priming induces a broad molecular reconfiguration that persists beyond the initial stimulus and may influence how clams respond to a later marine heatwave: RNA-seq evidence suggests that priming triggers a coordinated reprogramming of key pathways such as stress-response, energy metabolism and cellular protection mechanisms. At the chromatin level, ongoing analyses indicate that thermal priming reshapes regulatory accessibility and histone-mark landscapes at multiple genomic regions. These changes may provide a mechanistic explanation for the faster and more efficient response to thermal stress observed in primed animals. By moving from phenotype to gene regulation and chromatin dynamics, this work reframes thermal priming as an environmentally induced memory process with potential adaptive value in sessile and sedentary marine organisms. Understanding how such memory is encoded and maintained is essential to evaluate whether priming can be exploited as a practical mitigation strategy for shellfish aquaculture and conservation under increasingly frequent climate extremes.

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Back in black: recolonization and phenotypic adaptation challenge the evolutionary dead-end paradigm

Life-history specialization is often hypothesized to constrain evolutionary reversibility, potentially leading to evolutionary dead ends. Subterranean organisms exemplify this idea due to extreme adaptations such as eye loss and depigmentation. However, ecotones like springs (i.e., interfaces between caves and surface freshwaters) may offer opportunities to test whether highly specialized lineages retain phenotypic plasticity and evolutionary potential. After the discovery of spring populations showing signs of adaptation to surface of a freshwater isopod species (*Monolistra pavani*), currently considered as strictly cave-dwelling, we developed an integrative study. We combined population genomics, field surveys, spectroscopy and demographic modelling. Field surveys revealed stable spring populations composed of both pigmented and depigmented individuals, indicating that surface occurrence is not accidental. Spectroscopic analyses identified eumelanin-related absorbance peaks in pigmented spring individuals, supporting endogenous melanin production. Genome-wide SNP data showed strong genetic differentiation between cave and spring populations, with higher genetic diversity in springs. Approximate Bayesian Computation supported a historical simple split between cave and spring populations approximately 43,000 generations ago, rejecting scenarios of recent colonization or strong founder effects. Despite strong population structure, no loci under divergent selection were detected, suggesting limited adaptive genetic differentiation. Candidate-gene analyses identified a small number of pigmentation-associated loci related to melanogenesis, although these require functional validation. Palaeogeographic evidence indicates that caves could have acted as glacial refugia during the Late Pleistocene, whereas spring habitats became available after ice retreat. Together, our findings demonstrate that extreme specialization does not preclude the long-term exploitation of ecotones. The persistence pigmentation, coupled with population structuring but limited adaptive genomic differentiation, suggests that phenotypic plasticity may facilitate ecological transitions in highly specialized organisms. More broadly, our results highlight the potential for specialized organisms to retain unexpected evolutionary flexibility.

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To the end of the canal: morphology and evolution of the hindgut in the Apoidea

The alimentary canal of Hymenoptera extends through the entire body of the insect and is divided into three main regions: foregut, midgut and hindgut. Among these, the anterior and posterior portions exhibit considerable morphological diversification across insects, including Hymenoptera. Despite the biological importance of this structure, research to date has primarily focused on honey bees due to their economic importance, whereas the morphology and evolution of the gut in other Hymenoptera remain poorly investigated. Here, we investigated whether common ancestry influenced the evolution of selected anatomical features of the ileum and rectum, two portions of the hindgut, i.e. if these traits represent phylogenetic signals. Comparative histological analyses revealed subtle interspecific variation in both structures. Differences in the rectum involved the presence or absence, distribution, shape and size of the rectal pads, whereas variation in the ileum concerned cell nucleus position, cuticle thickness and cell shape. Phylogenetic relationships consistently and strongly account for the observed variation in these traits, indicating an important influence of common ancestry on hindgut morphology. Our findings suggest that hindgut morphology has evolved primarily under phylogenetic constraints and that can be thus used in morphological phylogenetic reconstructions. This study provides new and more comprehensive insights into the comparative morphology and evolution of the digestive system of Hymenoptera, helping to fill the gaps in our understanding of gut diversity through a broad histological approach. We encourage future comparative studies on digestive tract morphology across a wider range of hymenopteran taxa, also taking into account possible ecological drivers, such as nesting habit, diet specialization and social behaviour, that beside phylogeny influenced its evolution.

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Sensory pathway in aquatic insects: antennal sensilla and brain morphology during development

Aquatic insects represent a major component of arthropod diversity and dominate the fauna of many inland freshwater ecosystems. However, their sensory biology and neuroanatomy remain poorly understood. Over the last few years, we have investigated the antennal sensory pathway in representative taxa from three insect orders with aquatic immature stages, representing key evolutionary lineages of winged insects: Odonata (Palaeoptera), Plecoptera (basal Polyneoptera), and Trichoptera (Holometabola). Our initial studies described the antennal sensilla and primary olfactory centres of dragonflies and stoneflies, revealing common features of the aquatic sensory pathway despite the distant evolutionary relationships of these groups. In both orders, homologous brain structures process sensory information received through distinct antennal sensilla in aquatic juveniles and terrestrial adults, highlighting the developmental plasticity required in hemimetabolous insects to maintain sensory function throughout ontogeny. Recently, we extended this comparative framework by analysing antennal sensilla and brain morphology throughout postembryonic development in caddisflies, whose complete metamorphosis provides an opportunity to completely reorganize the sensory pathway from aquatic larvae to terrestrial adults. Our results reveal profound developmental changes associated with the transition from the aquatic to the terrestrial environment. Whereas larval antennae possess a relatively simple sensory equipment and a less differentiated deutocerebrum, adults exhibit a marked increase in sensillar diversity together with the development of well-defined antennal lobes and glomerular organization. Comparison across the three aquatic insect groups highlights both conserved features of the antennal sensory pathway in aquatic insects and lineage-specific adaptations related to ecology and metamorphosis. These findings provide a comparative framework for understanding the evolution of olfactory systems in insects and demonstrate how sensory structures and neural circuits are remodeled during development to meet the distinct ecological demands of aquatic immature stages and terrestrial adults.

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Manipulating minds: how parasitic trematodes reprogram fish brain and immune system

Parasites with complex life cycles often alter host behaviour in ways that increase transmission to subsequent hosts, yet the molecular mechanisms underlying these changes remain poorly understood. To investigate these processes, we used the strigeid trematode *Cardiocephaloides longicollis*, which infects the brain of gilthead seabream (*Sparus aurata*), an ecologically and economically important Mediterranean fish. Using our established *in vivo* model, fish were first experimentally exposed to the free-living larvae of *C. longicollis,* and later analysed at the early and mature stages of infection (approximately 7 days and 9 months post infection). Transcriptomic and proteomic analyses of brain tissue and cerebrospinal fluid revealed a pronounced temporal shift in host molecular responses. Early infection was characterized by suppression of innate immune pathways accompanied by activation of neuronal signaling processes, indicating an acute neuroimmune imbalance. In contrast, mature infection exhibited a distinct molecular profile characterized by persistent immune modulation, structural remodeling of neural tissue, and long-term regulation of gene expression associated with chromatin remodeling and post-transcriptional regulation, suggesting stabilization of infection-associated molecular programs. Integration of transcriptomic and proteomic datasets revealed consistent alterations in proteins involved in synaptic transmission, neuronal communication, and neuroimmune regulation. However, the most striking finding was not the alteration of individual pathways, but the progressive transition in the host response from early neuronal activation and immune suppression toward a stabilized molecular state during chronic infection. This temporal shift coincides with the transition of the parasite from an actively developing larval stage to a non-feeding encysted metacercaria. Despite this reduction in parasite activity, the host maintains a distinct molecular response, suggesting that infection progresses from an acute neuroimmune disturbance toward a persistent, stabilized state within the brain. Overall, our findings support a model in which trematode infection induces progressive neuroimmune reprogramming rather than a transient molecular response. This work provides new insight into host-parasite interactions in the vertebrate brain and identifies long-term molecular remodeling as a mechanism through which parasites influence host physiology and behaviour.

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Spiroplasma in *Glossina fuscipes fuscipes*: a potential manipulator of male reproduction

Glossina fuscipes fuscipes Newstead, 1910 (Diptera: Glossinidae), is a riverine species of *Glossina* (commonly known as tsetse flies) reported as the primary vector of Human African Trypanosomiasis and nagana in Uganda. These diseases are caused by the protozoan *Trypanosoma brucei*. As a tsetse fly, it displays a peculiar biology: adenotrophic viviparity as a reproductive strategy, where the female gives birth to a fully developed larva; a diet relying exclusively on vertebrate blood for both males and females; and a highly streamlined microbiota. Among the microbes characterizing their microbiota, *Wigglesworthia* represents the most intriguing, providing nutrients that the host cannot acquire through the strict diet, which are mandatory for the fly's development, survival, and reproduction. Furthermore, *Wigglesworthia* was also associated with anti-trypanosomic effects. Another intriguing bacterium was identified only in some populations of tsetse flies belonging to the Palpalis subgroup: *Spiroplasma*, which has already been associated with trypanosome refractoriness in *G. f. fuscipes*. We mainly investigated the effect of *Spiroplasma* on *G. f. fuscipes* male reproduction, focusing on mating fitness and molecular aspects, finding various impacts at different levels. We observed a modulation of mating behavior indicating non-random mating and an alteration of the cuticular hydrocarbon profile associated with the presence of *Spiroplasma*. Moreover, transcriptomic and proteomic analyses of *G. f. fuscipes* male reproductive organs revealed a significant negative influence of *Spiroplasma* on the testes, targeting processes related mainly to energy metabolism and sperm functionality. We also unveiled a potential alteration of the microbiota composition in the testes associated with the presence of *Spiroplasma*. Interestingly, among these bacteria, we found *Wigglesworthia* to benefit from the presence of *Spiroplasma*. In conclusion, this study provides new insights into the understanding of the *G. f. fuscipes*-*Spiroplasma* interaction. By modulating fly reproductive fitness and contributing to trypanosome refractoriness, *Spiroplasma* could be integrated into sustainable vector control.

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Tell me what you collect and I will tell you who you are: resource specialization is associated with phenotypic differentiation in honeybee foragers

Honeybee colonies rely on a sophisticated division of labour that allows workers to efficiently exploit resources and maintain colony homeostasis. While pollen foraging has been extensively investigated, resin collection remains comparatively understudied despite its crucial role in nest construction, social immunity and defence against disease. The present study aims to determine whether resin foragers constitute a distinct functional cohort within the foraging workforce and whether this functional specialization is associated with phenotypic differentiation in behaviour, in chemistry, of the microbiota and in immunity.

Emerging bees from three *Apis mellifera* colonies were uniquely tagged and monitored from 12 to 30 days of age using focal animal sampling (500 bees per colony). For each individual, the age at onset of foraging activity and every subsequent pollen- or resin-collecting trip were recorded during daily observation sessions (6 h day^{-1}). On day 30, identified pollen and resin foragers were collected for chemical, microbiological and molecular analyses.

Behavioural observations revealed clear task specialisation within the forager group and distinct differences in temporal polyethism. Individuals collecting resin repeatedly performed the same task throughout their foraging life, supporting the existence of a distinct cohort of specialised resin foragers rather than a homogeneous workforce. In addition, resin foragers exhibited a delayed temporal polyethism schedule compared with pollen foragers. Cuticular chemical analyses further supported this differentiation; resin foragers displayed a distinct cuticular chemical signature compared with pollen foragers, characterised by marked differences in hydrocarbon composition and the presence of specific compounds derived from collected resin. Analyses of the gut microbiota and of immune-related molecular pathways using 16S rRNA gene metabarcoding and RNA-seq respectively are currently underway and will provide further insight into the mechanisms underlying phenotypic divergence associated with foraging specialisation.

Overall, our results demonstrate that the resource collected by a worker is linked with distinct behavioural and chemical traits, highlighting an unexpected level of functional heterogeneity within the honeybee foraging workforce.

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Light and food as seasonal *zeitgeber* of the medaka circadian clock

The circadian clock is a key biological timing system that enables organisms to anticipate and adapt to daily environmental cycles. It consists of central and peripheral oscillators distributed across cells and tissues, which are entrained by environmental time cues, or *zeitgeber*. Under natural conditions, multiple *zeitgeber*, such as light and feeding, act simultaneously, and the circadian system must integrate these cues to maintain temporal organisation across seasons. Teleost fish provide valuable models for investigating seasonal plasticity of vertebrate circadian clocks, and the medaka (*Oryzias latipes*) is particularly suitable because of its pronounced photoperiodism and seasonality. In this study, we characterised circadian behavioural rhythms in medaka by examining entrainment to two major zeitgebers: light-dark cycles and food availability. We analysed photic entrainment of locomotor activity in larvae and adults under summer and winter photoperiods, using both full-spectrum and monochromatic light. We also assessed feeding entrainment in adults exposed to summer and winter conditions and tested behavioural responses when light and feeding cues were in conflict. Our findings show that both larval and adult medaka display daily behavioural rhythms that can be entrained by light-dark cycles and periodic food availability across seasonal conditions. Notably, light-feeding conflict experiments revealed seasonal differences in zeitgeber dominance: light was the main driver of behavioural entrainment under summer conditions, whereas feeding time appeared to dominate under winter conditions. In parallel, we used knockout mutants lacking functional *cpd*, *dash* and *6-4 photolyases* to investigate their role in circadian rhythmicity and sleep. Medaka mutants retained significant diurnal activity rhythms but showed reduced sleep compared with wild-type individuals. Overall, this ongoing study provides a behavioural characterisation of circadian rhythms in medaka, highlighting seasonal variation in the integration of photic and non-photic cues. Furthermore, it provides new insights into the role of photolyases in the regulation of circadian rhythmicity and sleep. Together, these findings establish a framework for future molecular studies aimed at elucidating the genetic and physiological mechanisms underlying light- and feeding-dependent entrainment of the circadian system.

Simposio 5

IMPATTI AMBIENTALI E STRATEGIE DI CONSERVAZIONE DELLA BIODIVERSITÀ



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Effects of habitat on the diversity and community composition of cleptoparasitic bees from rural protected areas in Latium (Italy)

Cleptoparasitic bees (cuckoo bees) are an ecologically important yet understudied component of wild bee communities, in particular their habitat preferences remain poorly understood. This study investigates how habitat affects their diversity and community composition in four protected rural areas of Rome province (Latium, Italy) under different management practices.

Bees were sampled monthly from March to October 2025 using entomological nets along fifteen transects (200×2m), distributed across three CORINE Land Cover classes: non-irrigated arable land, pastures, and land principally occupied by agriculture with significant areas of natural vegetation. Along each transect, phytosociological surveys were conducted. Microhabitat variables (bare ground, vegetation, debris and flower cover, and vegetation height) were recorded in four 1×1 m plots placed every 50 m along the transect. Landscape variables were photo-interpreted within a 250×250 m buffer around each transect to characterize the macro-habitat and summarized using Principal Component Analysis (PCA). Habitat effects on bee richness and abundance were assessed using Generalized Linear Mixed Models (GLMMs), whereas Redundancy Analysis (RDA) was applied to examine community composition. Floristic, environmental (microhabitat) and bee-community data were further analyzed through Hierarchical Cluster Analysis (HCA) to assess how sampling sites grouped together.

A total of 417 individuals belonging to 44 cuckoo bee species were collected. Preliminary results show that abundance and richness increase along the main landscape gradient, suggesting a positive effect of ecotonal complexity. At a finer scale, bare ground and flower cover positively affected both abundance and richness, while debris had a weak negative effect on abundance. Community composition was significantly structured by bare ground, flower cover and vegetation height, with several species showing clear associations with specific environmental variables, perhaps also important for their preferred hosts. The floristic classification identified three main vegetation groups, which appeared only partially congruent with bee-community clusters, whereas the environmental classification appeared more consistent with them.

These preliminary findings suggest that some macro- and micro-habitat variables play an important role in shaping the diversity and composition of cleptoparasitic bees, a known proxy for overall wild bee diversity.

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From fine-scale ecology to conservation planning: modelling range shifts in the butterfly *Papilio alexanor* under climate change

Insect decline is observed worldwide, with butterflies among the most studied and threatened groups in Europe. Besides habitat loss and degradation, climate change is increasingly recognised as a major threat. Many species are shifting their ranges towards higher elevations or latitudes. Identifying future climatically suitable areas is therefore a conservation priority, also considering specific regulatory obligations.

The emblematic *Papilio alexanor* is listed in Annex IV of the Habitats Directive, requiring Member States to achieve or maintain its favourable conservation status through strict protection. We investigated the potential effects of climate change on this species in Southern Europe, distinguishing two conservation units based on the two host plants used. Unlike most studies relying on generic species records, we used presence data restricted to pre-imaginal stages and specific to each host plant. This allowed a more accurate representation of the actual reproductive requirements of the species.

Moreover, future projections did not rely on climatic suitability alone but also incorporated landscape resistance and the dispersal capacity of the species, allowing us to identify the shift corridors most likely to ensure successful colonization. The two conservation units showed markedly different responses. The one linked to the plant typical of higher elevations is set to undergo a substantial range contraction, shifting towards higher altitudes. The other, linked to a more thermophilus plant, maintains a stable or expanding range, albeit with greater predictive uncertainty. The key conservation areas identified are already largely encompassed within existing Protected Areas.

These results provide useful guidance for conserving this species, supporting management differentiated by host plant. More broadly, they offer a framework for identifying priority areas and guiding the expansion of protected land under the EU Biodiversity Strategy 2030, also applicable to other species with well-defined ecological requirements.

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Avian diversity patterns over three decades provide new insights into the effects of *Amorpha fruticosa* invasion in riparian ecosystems

Riparian ecosystems are globally threatened by alien plant invasion, with *Amorpha fruticosa* representing one of the most critical cases in Europe. However, its impact on bird communities remains largely unknown. We addressed this knowledge gap by examining the effect of *A. fruticosa* invasion on riparian breeding bird assemblages within the Po River corridor (Northern Italy).

Bird communities were assessed at 86 point counts, while environmental variables were collected within a 100-m buffer around each point count. We tested the association of taxonomic and functional diversity metrics with *A. fruticosa* cover, accounting for landscape composition. Historical surveys available for the same area enabled us to describe long-term (33 years) changes in bird community composition.

Since 1992, 31 species have declined in occurrence, while 18 have increased. *A. fruticosa* cover positively affected species richness, community conservation index and the functional dispersion, suggesting that this non-native plant may act as a surrogate habitat for shrubland specialists. However, *A. fruticosa* spread could have contributed to the local decline of open-habitat species. While forest cover and landscape compositional heterogeneity promoted species richness, heterogeneous and degraded habitats – both wooded and herbaceous – reduced specialisation level and functional evenness of avian communities.

Management strategies should prioritise the restoration of native seminatural grassland and the conservation of well-preserved woodlands. At the same time, the eradication of *A. fruticosa* should be carefully evaluated, as it may negatively impact the relict populations of several avian species of conservation concern and could be largely unfeasible due to its pervasive distribution.

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Environmental adaptation and species divergence in the genus *Chamelea* (Mollusca: Bivalvia): evidence from genome-wide SNP data

The genus *Chamelea* includes ecologically and economically important bivalves distributed across the Mediterranean Sea, Black Sea and adjacent Atlantic waters. However, the relative contribution of environmental adaptation and species divergence to the evolutionary dynamics of these taxa remains poorly understood. We investigated genome-wide patterns of genetic diversity, adaptive variation and species divergence within the genus *Chamelea* using ddRAD-seq data from 440 individuals sampled across the Adriatic Sea, Mediterranean Basin, Black Sea and Atlantic Ocean. Population genomic analyses based on 9,083 unlinked SNPs revealed a clear genetic separation between *C. gallina* and the closely related Atlantic species *C. striatula*, supporting their status as distinct species. Within *C. gallina*, populations displayed weak but geographically meaningful structure. Adriatic populations formed a largely homogeneous genetic cluster, whereas western Mediterranean, Atlantic and Black Sea populations showed significant differentiation. To investigate adaptive processes, genomic data were integrated with environmental variables through Gradient Forest and Latent Factor Mixed Model analyses. Sea surface temperature and salinity emerged as the main predictors of adaptive genomic variation, suggesting that environmental selection contributes to maintaining adaptive differentiation despite limited neutral genetic differentiation. Shell microstructure was additionally investigated through scanning electron microscopy. In contrast to genomic patterns, shell organization showed marked variability both within and among populations, supporting a major role of phenotypic plasticity in biomineralization processes. Our results indicate that environmental heterogeneity and adaptive divergence play a major role in shaping the evolutionary dynamics of *Chamelea* populations. The integration of genomic, environmental and phenotypic information provides new insights into the adaptive potential of marine bivalves and highlights the importance of combining multiple levels of biological organization when investigating responses to environmental change.

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From short-term experimental manipulation to long-term removal of managed honey bees: six years of research and conservation actions to understand trophic competition and wild bee population trends on Giannutri island

Whether managed honey bees (*Apis mellifera*) negatively affect wild bee populations through trophic competition has been debated for more than two decades. Evidence remains controversial because competitive effects strongly depend on ecological context, and few studies have simultaneously assessed resource depletion, behavioural responses, and long-term population trends.

In 2021, we initiated a long-term study on Giannutri Island (2.6 km², Tuscan Archipelago, Italy), where an apiary of 18 managed honey bee colonies provided a unique opportunity to investigate these interactions at the scale of an entire island. During 2021 to 2024, we combined standardized population monitoring of wild bees with experimental short-term honey bee exclusion and measurements of nectar and pollen availability. Our results showed that temporary honey bee removal increased floral resource availability and improved wild bee foraging efficiency. Over the same period, populations of the two focal species, *Anthophora dispar* and *Bombus terrestris*, declined by approximately 80%, a pattern consistent with strong trophic competition from managed honey bees.

Based on these findings, the Tuscan Archipelago National Park decided not to renew the permits for managed beekeeping on the island after the 2024 season. This management decision unexpectedly transformed our experimental system into a long-term natural experiment. Monitoring and behavioural observations continued throughout 2025 and 2026 under complete honey bee absence. Preliminary results indicate a marked increase in wild bee foraging efficiency together with a rapid recovery of both focal populations, whose abundances increased by approximately 400 to 600% compared with 2024. These findings further support the hypothesis that managed honey bees reduced wild bee fitness through exploitative competition for floral resources.

The continuation of this study will provide a rare opportunity to disentangle the respective contributions of managed honey bees, natural population fluctuations and annual climatic variability in shaping long-term population dynamics of wild bee communities.

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Evaluating vertebrate diversity in a Sicilian fluvial ecosystem: an environmental DNA biomonitoring study of the Oreto river

Freshwater ecosystems on Mediterranean islands represent crucial evolutionary refuges; however, they are subjected to intense, synergistic anthropogenic and climatic pressures while remaining largely understudied. The Oreto River (Sicily, Italy) represents a prime example of such a highly stressed peri-urban lotic system. In this study, environmental DNA (eDNA) metabarcoding targeting the mitochondrial 12S rRNA gene was applied for the first time across three stations (Upstream, Midstream, and Downstream) to provide a comprehensive, multi-taxa preliminary screening of vertebrate diversity—encompassing fish, amphibians, birds, and mammals—along the entire longitudinal gradient of the river.

The genetic data revealed spatial variations in taxonomic composition and beta-diversity that closely mirror localized land-use pressures along the river continuum. In the upstream reach, the dataset yielded a predominantly "Wild" faunal signature, including the potential presence of the highly threatened Mediterranean trout (*Salmo cettii*), which would represent the first putative evidence of this endemic salmonid in North-Western Sicily. In the midstream reach, the eDNA profile transitioned significantly, becoming dominated by "Agricultural and Domestic" genetic signatures (e.g., *Bos taurus*, *Ovis aries*, *Sus scrofa*), reflecting a strong agricultural and pastoral footprint along the middle corridor. Finally, the downstream reach near the river mouth revealed a complex, estuarine-like assemblage; notably, it captured sequences assigned to the critically endangered European eel (*Anguilla anguilla*), but within a context highly characterized by non-native and invasive taxa (e.g., *Ameiurus melas*, *Cyprinus carpio*, *Squalius squalus*, *Tinca tinca*). Crucially, putative genetic traces of marine commercial fish were detected, pointing to the confounding influence of urban wastewater discharges and food-waste runoff.

Ultimately, spatial ordination (PCoA) mathematically confirmed a sharp longitudinal degradation of the river ecosystem. The strong congruence with historical local surveys demonstrates that this approach provides a robust, non-invasive screening framework that holds significant potential for guiding conservation and monitoring strategies in threatened Mediterranean island basins.

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Soil biological quality and biodiversity at the margins of rice fields: insights from QBS-ar and eDNA metabarcoding

Agricultural intensification and landscape homogenization have driven a widespread decline in agroecosystem biodiversity. Rice field margins serve as surrogate habitats for non-wetland edaphic species. However, conventional agricultural practices can degrade these margins, and the relative beneficial effect of organic management remains poorly understood.

We combined the Arthropod-based Biological Soil Quality Index (QBS-ar), which assesses soil biological quality through the analysis of microarthropod communities and their morphological adaptations to hypogean life, with an environmental DNA (eDNA) approach to characterise soil metazoan communities. This integrated approach was used to evaluate the effects of paddy fields management on soil biodiversity of field margins. We hypothesised that different management practices inside the ground of paddy fields impose different levels of pressure on soil biodiversity even in rice field margins.

We studied a lowland agroecosystem located in the provinces of Vercelli and Novara (NW Italy). A total of 108 soil samples were collected in two monitoring seasons (spring and summer) across two crop systems (organic and conventional) and a natural habitat located in three distinct areas. Within each system, two field margins belonging to three different rice paddies were sampled. In the natural control habitat, we selected six points on the margin of three ponds. The QBS-ar was determined for each soil core. For eDNA, libraries construction involved the amplification of 313 bp region of the metazoan COI gene and subsequent bioinformatic analysis inferring ASVs using the DADA2 R package and a tailored reference database.

Both methods revealed that invertebrate communities of the margins did not differ significantly between organic and conventional crop systems, contrary to known in-field patterns. However, both agricultural systems differed significantly from natural habitats. The eDNA provided a higher taxonomic resolution, identifying indicator species sensitive to management effects. These findings suggest that field margins in both rice systems suffer from similar disturbances, overriding the potential benefits of organic cultivation. Integrating morphological indices like QBS-ar with high-resolution eDNA metabarcoding offers a robust framework for agroecosystem biomonitoring, highlighting the important role of natural wetlands as biodiversity reservoirs.

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Gene expression response to environmental intoxication in stick-insects *Bacillus rossius*

We investigated differential gene expression in a clonal line of the Mediterranean stick insect *Bacillus rossius* (Rossi, 1790) bred under controlled condition and exposed to airborne pollutants deposited on leaves of *Rubus* sp. from two industrially polluted sites in Civitavecchia and Rome, and a control site near “Saline di Tarquinia” reserve. For this purpose, insects were kept under uniform conditions of climatic chambers for 12 days to assess the effect of environmental intoxication mediated by dietary intake of *Rubus* sp. leaves. After exposure, total RNA from Malpighian tubules (M) and mid-posterior gut (G), as excretive/detoxifying organs, was extracted and sequenced. Reads were mapped to the annotated *B. rossius redtenbacheri* reference genome. Differential gene expression and functional enrichment analyses were performed using R with DESeq2 package.

A total of 14,389 genes were mapped, and low counts filtered before differential expression analysis. Only 10 differentially expressed genes (DEGs) were detected per tissue between the two polluted sites, hence treated as a single experimental group due to the highly similar response. Compared with the control group, however, 37% and 40% DEGs were found in the M and G, respectively, including both up- and down-regulated genes. Cluster analysis revealed two clusters among DEGs in both tissues, corresponding to up- and down-regulated genes in the experimental group versus control. Overrepresentation analysis (ORA) of KEGG pathways and gene ontologies (GO) terms was performed for each cluster, while gene set enrichment analysis (GSEA) was applied to the full set of analysed genes. Overall, ORA and GSEA provided complementary insights. Genes and pathways associated with apoptosis, glycerolipid metabolism, multiple signalling cascades (FoxO, cadherin, IgSF CAM, etc.), cytoskeleton dynamics and DNA-binding were up-regulated, and those related to oxidative phosphorylation and electron-transport chain elements, carbon metabolism, translation, and ribosome, proteasome, spliceosome and peroxisome metabolic pathways – down-regulated.

Results suggest that *B. rossius* exhibited signs of physiological stress, potentially attributable to environmental contaminants inducing oxidative stress, such as heavy metals, although further chemical analysis of the leaves is scheduled to identify the causative compounds. These findings support the use of *B. rossius* as a sentinel species for air pollution biomonitoring.

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Drivers of dead wood colonization by ants in temperate forest

Deadwood is a key structural component of forest ecosystems, yet the role of ants in saproxylic communities has received comparatively little attention. Here, we examined ant colonization across 5,826 deadwood fragments of *Quercus* spp. and *Castanea sativa* in a temperate woodland of Northern Italy. We assessed the effects of wood characteristics (diameter, length, hardness, decay class), tree species, topographic variables, and artificial fissuring on colonization probability, colony composition, and interactions with other saproxylic taxa.

Ants were found in 39.1% of *Quercus* spp. and 13.4% of *C. sativa* fragments, with colonized pieces larger in diameter and length. The effects of hardness and decay class were species-specific: colonized oak fragments tended to be harder, while colonized chestnut fragments were softer and more decayed. Colony composition differed markedly among the 13 ant species recorded, with incipient and mature colonies occupying distinct microhabitats. *Temnothorax alienus* and *T. unifasciatus* were strongly associated with complete colonies, while *Lasius emarginatus* and *Crematogaster scutellaris* were more frequently associated with founding queens or early-stage colonies. Multivariate generalized linear model analyses revealed significant effects of tree species, topographic parameters, and deadwood characteristics on community composition. Artificial fissuring substantially increased colonization by both ants and saproxylic beetles.

Taken together, our results show that ants are selective colonizers of deadwood, responsive to fine-scale structural and microenvironmental variation, and play a part in shaping saproxylic assemblages. These findings provide empirical support for forest management approaches that incorporate deadwood retention and acknowledge ants as functionally important agents within decomposing wood.

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Essential oil-based smart formulations processed by plasma: evaluation of their effects on soil nematode communities

Root-knot nematodes, particularly *Meloidogyne incognita*, constitute a major threat to horticultural crops, while the restriction of synthetic nematicides highlights the need for effective and more eco-compatible strategies. In this context, essential oil (EO)-based formulations carried on zeolite offer a promising alternative; however, their environmental impact must be assessed in relation to non-target soil biodiversity.

This study evaluates the response of free-living nematode communities, used as bioindicators of soil ecological quality, in a pot experiment with formulations based on three EOs: *Allium sativum*, *Cinnamomum verum*, and *Ruta graveolens*. EOs were loaded onto zeolite granules, selected as a porous carrier for controlled release, and tested uncoated or with different barrier coatings obtained by plasma deposition. Soil sampling followed a BACI approach, before granule application and after approximately 17 and 65 days. Formulations were compared with untreated controls and a conventional chemical nematicide. Free-living nematodes were extracted, identified at genus level, and assigned to trophic groups and colonizer-persister (c-p) guilds. Taxonomic and functional community responses were assessed through ecological indices and multivariate analyses.

Overall, the analysis indicated that changes in free-living nematode communities were mainly associated with temporal dynamics and experimental conditions rather than with marked treatment-specific effects. The tested EO-based formulations did not show clear evidence of strong adverse impacts on beneficial soil nematode assemblages under the adopted experimental conditions. These findings suggest that controlled-release EO formulations may represent a promising approach for plant-parasitic nematode management, while reducing potential disturbance to functional soil biodiversity. Further field investigations are needed to confirm their effectiveness and ecological compatibility in more complex agroecosystems.

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Demographic estimation and conservation guidelines for *Coenagrion castellani*: insights from a 10-year study in an agricultural landscape

Coenagrion castellani, an Italian endemic damselfly included in Annex II of the Habitats Directive, is one of the target species of the LifeIMAGINE project (LIFE19 IPE/IT/000015). Since 2017, its adult population has been monitored throughout the flight seasons in an intensively cultivated agricultural landscape in Central Italy. Over a ten-year period, approximately 6,000 adults were captured, marked, and recaptured, generating a long-term dataset that enabled the estimation of apparent survival (ϕ) and detection probability (p).

Building on this unique dataset, we developed a Bayesian power-analysis framework based on the Cormack–Jolly–Seber (CJS) model to identify efficient monitoring strategies for this species. In particular, we evaluated the effects of three fundamental components of capture-mark-recapture (CMR) study design on the precision of demographic estimates.

This framework provides practical guidance for optimizing CMR monitoring protocols by maximizing the precision of demographic estimates while minimizing field effort. It also supports the development of evidence-based conservation actions, including management guidelines and a drainage ditch maintenance plan for sites occupied by *C. castellani*. Together, the optimized monitoring strategy and the associated management recommendations provide a robust scientific basis for assessing the species' conservation status and promoting its effective in situ conservation.

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Species-specific temporal responses to urbanization in Italian urban mammals

Urbanization is one of the most pervasive anthropogenic pressures affecting wildlife worldwide and can profoundly alter animal behavior. Among behavioral traits, diel activity patterns are increasingly recognized as flexible components of species' ecological niches that can shift in response to environmental and human-related factors. However, the effects of urbanization on mammalian temporal behavior remain poorly understood, particularly in Mediterranean urban ecosystems.

We investigated diel activity patterns and temporal niche selection of medium- and large-sized mammals using an extensive camera-trap dataset collected across 58 sites distributed in five Italian cities characterized by different levels of urbanization, i.e., Rome, Milan, Florence, Naples, and Campobasso. In 2024 and 2025 we collected over 18,000 photographs and videos of 12 wild mammal species.

We employed a temporal resource selection framework to evaluate selection for nocturnal, diurnal and twilight periods. Additionally, we used trigonometric generalized linear mixed models (GLMMs) to analyze fine-scale shifts in activity peaks across the 24-hour cycle and to evaluate the influence of environmental and anthropogenic variables. These latter include land-cover composition, imperviousness, tree cover density, human population density and local human activity. Results revealed marked interspecific differences in temporal responses to urbanization. Red foxes (*Vulpes vulpes*) showed increased selection for nocturnal activity in highly urbanized areas while maintaining a relatively stable bimodal activity pattern. Wild boars (*Sus scrofa*) exhibited both stronger nocturnal selection and significant changes in activity curves along urbanization gradients, suggesting substantial behavioral adjustments to anthropogenic environments. In contrast, European badgers (*Meles meles*) showed no detectable effects of urbanization on either temporal selection or activity patterns, maintaining consistent behavior across study sites.

These findings suggest that urban mammals adopt distinct temporal strategies to cope with anthropogenic landscapes, ranging from behavioral stability to substantial temporal niche reorganization. Our study highlights the importance of considering the temporal dimension of ecological niches when investigating wildlife adaptation to urban environments and provides new insights into the mechanisms promoting species persistence in urbanized ecosystems.

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Metabarcoding beyond morphology in the CRIMAC BluCaProd project: uncovering hidden meiofaunal diversity along the Calabrian Ionian coast

Meiofauna is a highly diverse and functionally important component of benthic ecosystems. The small size of these organisms, their short life cycles, sediment dependence and sensitivity to environmental changes make them valuable indicators of coastal ecosystem health. In the frame of a wider project (CRIMAC BluCaProd), we assessed meiofaunal biodiversity along the Calabrian Ionian coast, a poorly studied Mediterranean area exposed to multiple anthropogenic pressures, including riverine inputs, tourism, trawling and urbanization. Sediment samples were seasonally collected in 2023 at nine coastal stations located off the coasts of Amendolara, Sibari, Corigliano, and Calopezzati, at 5 m and 15 m depths, along with an offshore station off the Amendolara Shoal, at 25 m depth. Morphological identification of taxa was integrated with metabarcoding-based molecular profiling and alpha-diversity analyses.

Optical microscopy investigations revealed heterogeneous assemblages dominated by Nematoda and Copepoda, with additional contributions of Platyhelminthes, Ostracoda, Polychaeta, Mollusca, Foraminifera, and Tardigrada. Taxonomic richness increased in autumn, reaching 25 morphotaxa in Sibari and 24 in Corigliano. Molecular analyses substantially expanded our biodiversity estimates, detecting over 2,200 amplicon sequence variants assigned to 48 phyla, 186 orders, 279 genera, and 553 species. This approach improved the detection of soft-bodied, fragile, and cryptic taxa, including Platyhelminthes, Gastrotricha, Xenacoelomorpha, Nemertea, Rotifera, and Annelida. Diversity indices highlighted spatial and seasonal variability: Chao1 richness peaked in Corigliano and Calopezzati, whereas Shannon and Simpson indices indicated the most uniform communities in Amendolara, particularly in winter. The lowest diversity values occurred at Sibari, suggesting a stressed assemblage.

This study represents the first assessment of meiofaunal diversity in this area, which is exposed to multiple anthropogenic pressures, and will lay the groundwork for the development of tools for environmental management. Overall, the results of this study provide the first comprehensive meiofaunal assessment of this coastal sector and demonstrate that integrating morphology and metabarcoding may result in a high-resolution approach for biodiversity monitoring. Meiofauna emerges as a sensitive indicator of environmental quality and a valuable tool for the management of Mediterranean coastal ecosystems.

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Effects of olive mill wastewater on sea urchin *Arbacia lixula* (Linnaeus, 1758)

Olive mill wastewater (OMW), the main by-product from olive oil production, represents a significant environmental concern in the Mediterranean basin. It is typically released into the environment through direct discharge into sewage systems and natural watercourses, or via runoff following land application and evaporation pond storage. OMW constitutes a serious ecological threat due to its high organic load, strong acidity, and elevated concentration of phenolic compounds. Although its toxicity has been documented in several aquatic organisms, its effects on marine species remain largely underexplored. Among Mediterranean marine organisms, the sea urchin *Arbacia lixula* (Linnaeus, 1758) is a benthic herbivorous species of great ecological relevance, widely used as a bioindicator of coastal environmental conditions.

This study evaluates the cellular responses of this species to olive mill wastewater (OMW). Initially, a 96-hour acute toxicity test was conducted across a range of OMW concentrations (0.5%, 1%, 2%, and 4%) to determine lethality thresholds, yielding an LC_{50} value of 0.63%. Based on these findings, subsequent 96-hour sublethal exposures were carried out at concentrations corresponding to 1/10 and 1/20 of the LC_{50}. Following the exposure period, cellular stress biomarkers were quantified in the stomach, intestine, gonads, peristome, and celomatic fluid of *A. lixula* using protein- and enzyme-based assays.

Preliminary results reveal a marked and tissue-specific response, characterized by a significant modulation of the analysed biomarkers according to the biological compartment. Specifically, these alterations reflect a differentiated impairment of cellular defence systems and redox mechanisms across the examined tissues.

Taken together, the results indicate a heterogeneous susceptibility of each tissue compartment to sublethal OMW exposure, underlining that the activated biological mechanisms are highly tissue dependent. This integrated approach underscores the importance of expanding ecotoxicological testing to a broader range of coastal species to fully characterize their distinct biological responses. Such a framework could be essential for establishing accurate discharge limits for industrial effluents and developing sustainable management strategies based on marine biodiversity.

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New insights into the distribution, habitat preferences, and life-history traits of *Eriogaster catax* in Umbria (Italy), with implications for conservation management

The moth *Eriogaster catax,* listed in Annex II and Annex IV of the European Union Habitat Directive (92/43/EEC), was studied to enhance knowledge of its distribution, ecological characteristics, habitat requirements, and life-history traits in the Umbria region, Italy. This work supports conservation actions under the LIFE IMAGINE UMBRIA project (LIFE19 IPE/IT/000015). We updated *E. catax* regional distribution through bibliographic review and field surveys; the database increased from 17 records (1994-2017) to 123 by 2024. *E. catax* is now documented in 17 10×10 km squares (89% increase) and in 12 Natura 2000 sites (71% increase). To better understand the habitat requirements of this species in the territory of Umbria region, we analysed oviposition site selection by recording host plant species, oviposition height, shrub height, and branch diameter.

Egg batches and larval nests occurred almost exclusively on *Prunus spinosa*. Mean oviposition height was 74 cm (range 20–175 cm), with 41–60 cm most used. Shrub height averaged 124 cm (range 60–300 cm), with 51–100 cm most frequent. Branches used for oviposition averaged 5 mm (range 2–10 mm). Most plants hosted a single larval nest. We conducted an *ex-situ* rearing study using field-collected egg batches to investigate the species life-history traits and phenology under laboratory conditions closely replicating its natural environment. Body length, head capsule width, and weight were recorded across larval instars. Average larval length and head width increased from 4.29 mm and 539 µm (instar I) to 38.97 mm and 3117 µm (instar V), with average weight increasing from 0.0018 g to 0.7206 g. Development from hatching to pupation averaged two months. Pupae were measured, weighed, and individually aestivated. Adult emergence occurred from 9–27 October for females and 16 October–6 November for males, with mean adult weights of 0.2199 g and 0.1414 g, respectively.

These biometric and rearing data complement field observations on habitat requirements and ecological preferences, enhancing understanding of *E. catax* biology and ecology in Umbria and providing evidence to guide conservation and habitat-management measures.

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Soil nematodes as bioindicators of agroecosystem health: evaluating essential oils in a One Health context

From a One Health perspective applied to agroecosystems, soil health represents a key link between biodiversity, ecosystem functioning, and the environmental sustainability of agricultural systems. In this context, soil free-living nematodes are particularly useful bioindicators, as they occupy multiple trophic levels and reflect the structure and functioning of soil food webs. In organic farming, essential oils may contribute to a more sustainable management of crop pests, but their evaluation should not be limited to their efficacy against target organisms: potential impacts on non-target soil organisms must also be considered.

This study evaluated rosemary (*Salvia rosmarinus*), lavender *(Lavandula angustifolia*), and thyme (*Thymus vulgaris*) essential oils in an organic horticultural system in Central Italy, considering both their efficacy against leafminer flies and their potential ecological effects on soil free-living nematodes. Field trials were carried out on chard and spinach over two growing seasons, using different essential-oil concentrations: 0.05% in the first year and adjusted concentrations in the second year, selected according to preliminary phytotoxicity tests. Two leafminer species were recorded, *Liriomyza bryoniae* and *Pegomya cunicularia*. Essential oils showed variable efficacy, mainly limited to specific phases of the crop cycle. Within this integrated assessment, particular attention was given to the nematode community, used as an indicator of soil ecological quality and treatment sustainability. Soil samples were collected before treatment application and after 7 and 30 days. Nematodes were identified to family or genus level, assigned to trophic groups and colonizer-persister guilds, and analysed through diversity and functional indices. Temporal dynamics were the main driver of nematode community variation. However, higher essential-oil concentrations produced measurable effects: lavender reduced alpha diversity and simplified the soil food web, thyme induced intermediate responses, whereas rosemary caused no clear alterations.

These results suggest that free-living soil nematodes may support the sustainable evaluation of essential oils, linking pest management, soil ecosystem functioning, and environmental health within a One Health framework.

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Multifaceted effects of honeybee hive proximity on an endemic, insular wild solitary bee

It is increasingly suggested, and in few cases experimentally proven, that managed honeybees (*Apis mellifera*) have a detrimental impact on wild bees, primarily because of the heavy competition for floral resources (pollen and nectar) driven by very high local hive abundances, especially in the vicinity of apiaries. In the delicate, often resource-limited island ecosystems, such negative effects may concern endemic wild bee species with high conservation value. However, studies on the effects of honeybees on insular bee species are still very limited.

Here, we investigated in a two-year study whether proximity to honeybee apiaries affect the demography and phenotypic traits of the endemic solitary bee *Hylaeus canariensis* in the protected high-elevation ecosystem of the Teide National Park in Tenerife (Canary Islands). Using a well-established trap-nesting protocol to allow *Hylaeus canariensis* nesting and rearing its brood along a gradient of increasing distance from apiaries, we evaluated shifts in reproductive success, sex-ratio, body size, visual system and cuticular hydrocarbon (CHC) profile. While we found evidence for greater productivity or – depending on the year – female-biased sex-ratio closer to the apiaries, we have also detected a decrease in nest cell completion rate at higher density of active hives. Additionally, wild bees emerging closer to apiaries exhibited smaller compound eyes relative to body size, narrower and (marginally) smaller wings, and lower functional trait richness. Finally, females closer to the apiaries showed less complex CHC profiles characterized by fewer methyl-branched alkanes with shorter chain lengths.

These results suggest that managed apiaries in the study area may constitute a disturbing factor for this endemic wild bee species, potentially influencing both their demography and their phenotype. These findings underscore the need to carefully regulate apiculture practices within protected areas on islands.

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Natural CO_2 mofettes as field models of chronic hypercapnic stress: integrating ecotoxicology and soil biodiversity responses

Soil arthropods are central actors in extreme ecosystems, like mofette ones, where long-term geogenic CO_2 emissions reshape the chemical and biological template of the soil. These rare terrestrial systems provide natural laboratories for examining how belowground faunal communities respond to chronic hypercapnic conditions, offering analogues for future scenarios with increasing atmospheric and soil CO_2 concentrations. We investigated arthropod responses along a natural CO_2 gradient in the Bossoleto mofette (Tuscany, Italy), comparing four habitats: reference grassland (REF), *Agrostis stolonifera* slopes (AS), *Phragmites australis* bottoms (PB), and unvegetated bottom soils (BB). Soil physicochemical properties were assessed together with active and passive indicators, including assays with *Folsomia candida*, *Porcellionides pruinosus* and *Lepidium sativum*, native arthropod communities, QBS indices, and collembolan traits. The four habitats differed markedly in soil chemistry, defining a gradient from neutral and less carbon-rich REF soils to wetter, more acidic, and carbon-enriched PB and BB soils.

Soil pH and carbon-related variables emerged as the principal environmental filters structuring arthropod responses. Significant effects were detected in *F. candida* reproduction and growth and in the avoidance behaviour of *P. pruinosus*, whereas plant responses were less sensitive. Community descriptors showed a clear decline from REF and AS to PB and BB habitats, with reductions in arthropod richness, Shannon diversity, and soil biological quality. Multivariate analyses consistently separated arthropod assemblages from reference and mofette-bottom habitats. Soil pH was the main factor associated with community turnover, while trait-based analyses revealed the dominance of highly edaphic and morphologically specialized collembolan forms under extreme mofette conditions. The integration of ecotoxicological, biodiversity, trait-based, and soil chemistry data highlighted a progressive reorganization of soil arthropod communities along the hypercapnic gradient.

Our findings demonstrate that long-term geogenic CO_2 exposure reshapes belowground faunal communities primarily through persistent modifications of soil properties rather than direct toxicity alone. Natural mofettes therefore represent valuable model systems for understanding how soil arthropods respond to chronic CO_2 enrichment and for predicting belowground biodiversity shifts under future environmental change.

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Stress in rearing: biochemical and growth impacts of acoustic exposure in juvenile *Dicentrarchus labrax*

Acoustic pollution is an emerging concern in aquaculture, where continuous exposure to noise can affect the welfare of farmed fish. Juvenile fish may be more susceptible to acoustic disturbances during their growth phases, which could potentially affect their physiological responses. The European seabass (*Dicentrarchus labrax*) is an important species in aquaculture due to its economic value and widespread farming, making it a relevant model for studying the effects of acoustic pollution in aquaculture environments.

This study aimed to evaluate the short-term effects (30 minutes, 3 hours, 24 hours, and 7 days) of traffic noise exposure on the cellular responses of juvenile *Dicentrarchus labrax*, using whole-body analyses. Acoustic emissions were recorded from four different fishing vessels at distances between 10-15 meters, with recordings emitted randomly and with 10 to 30-second intervals of silence between consecutive emissions. The biomarkers tested included heat shock protein 70 (HSP70), superoxide dismutase (SOD), glutathione peroxidase (GPx), adrenocorticotropic hormone (ACTH), cortisol, glucose, esterase, and alkaline phosphatase.

The results revealed significant alterations in these parameters after just 30 minutes of exposure, with a tendency toward recovery after 7 days. Furthermore, a statistically significant reduction in weight relative growth rate was observed in exposed individuals. Although these findings may not apply to all species of farmed fish, they provide valuable insights into minimizing acoustic impacts and improving animal welfare in aquaculture environments.

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Effects of nanoplastics on cell-mediated immune responses of ascidians

Plastic contamination has become one of the most pervasive environmental challenges affecting marine ecosystems. Among plastic-derived pollutants, nanoplastics (NPs) are of particular concern because of their small size, long-term persistence, and high bioavailability, which facilitate their interaction with biological tissues and cells. Filter-feeding marine invertebrates, including ascidians, are especially vulnerable to NP exposure due to their continuous filtration of large volumes of seawater. Nevertheless, the impact of nanoplastics on the cellular immune system and physiological functions of these organisms remains largely unexplored.

In this study, we investigated the *in vitro* effects of polyethylene nanoplastics (nPET; 0.5 and 5 µg/mL) on *in vitro* phagocytic activity of circulating haemocytes from the colonial ascidian *Botryllus schlosseri* and the solitary ascidian *Ciona robusta*. We also evaluated the degranulation of cytotoxic haemocytes (morula cells) as a consequence of the recognition of *Bacillus clausii* cells. Animals were subjected to different exposure protocols. *B. schlosseri* colonies were either exposed for 7 days to nPET in filtered seawater (FSW) at the selected concentrations or injected with nPET under four experimental conditions: primed (injections on days 1 and 7 and assays on day 8), treated (injection on day 1 and assays on day 2), injected control (vehicle injection on day 1 and assays on day 8), and non-injected control. Conversely, *C. robusta* individuals were exposed for 7 days to 5 µg/mL nPET in FSW.

Our findings suggest that cytotoxic haemocytes are more responsive to nPET exposure than phagocytic cells and their activation depend on the exposure regime in both ascidian species. Conversely, the phagocytic activity of *B. schlosseri* haemocytes was not significantly altered by nPET treatment, whereas *C. robusta* seems to show enhanced phagocytic activity following nPET exposure. These results highlight species-specific differences in immune responsiveness to NPs and support the use of ascidians as valuable models for investigating the biological effects of emerging pollutants. Future work will focus on elucidating the molecular mechanisms underlying the immune behaviour and on further exploring the consequences of NP exposure for ascidian immunity, development, and physiology.

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Breaking the silence: anthropogenic noise pollution and its impact on Cephalochordates

In recent decades, escalating human activities have profoundly altered marine soundscapes, emerging as a pervasive threat to ecosystems. While anthropogenic noise detrimentally impacts diverse marine life, bioacoustics research has historically focused predominantly on vertebrates. Consequently, invertebrate bioacoustics and their behavioral and physiological responses to noise remain poorly understood. Cephalochordates (amphioxus) offer a unique perspective on this understudied field. Possessing vertebrate-like anatomy and sensory cells homologous to vertebrate hair cells, they represent an optimal model for investigating acoustic pollution.

To address this gap, our study investigated the multi-level effects of marine noise on *Branchiostoma lanceolatum*, integrating oxidative stress analysis, RNA-sequencing transcriptomics, and filter-feeding behavior. In a controlled tank, amphioxus specimens were exposed for one hour to two calibrated maritime traffic soundtracks: a real-world underwater recording and a synthetic noise track generated in accordance with the Marine Strategy Framework Directive guidelines. We measured their responses at two points: immediately after the noise exposure and following a 24-hour recovery period.

Our data provide definitive evidence that amphioxus actively perceives acoustic stimuli. Furthermore, even short-term exposure triggers significant physiological, molecular, and behavioral alterations, highlighting the urgency of expanding marine noise research and policies to protect vulnerable invertebrates.

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Temporal genomics reveals erosion of functional genetic diversity in the Atlantic bluefin tuna

Atlantic bluefin tuna (*Thunnus thynnus* Linnaeus, 1758) is a large, highly migratory predator supporting long-standing fisheries in the Mediterranean Sea and the eastern Atlantic Ocean. Stock assessments and rebuilding targets often rely on recent catch and survey series, potentially masking long-term biodiversity loss through shifting baselines. We used a temporal dataset of high-coverage genomes to quantify changes in genetic diversity across the last millennium and to test whether the claimed twentieth-century collapse left a detectable imprint on genetic diversity variation within the eastern Atlantic stock.

We analyzed 41 ancient (from 3000BCE to 1941) and 49 modern (2013-2018) individuals under a strictly symmetric filtering framework and compared genome-wide diversity and the distribution of variants across functional genomic regions. Modern tunas show a modest but significant reduction in genome-wide diversity relative to ancient ones (approximately 1-2% reduction), consistent with a historical demographic decline that may have started before the pronounced twentieth-century collapse indicated by fishery-dependent data. However, changes were not uniform across the genome: diversity shifts were relatively stronger in functionally constrained regions than in putatively neutral portions of the genome, indicating a disproportionate erosion of variation likely to contribute to fitness and adaptive potential.

Our results provide an independent, fishery-complementary line of evidence for recent biodiversity erosion and help reconstruct pre-collapse baselines. Integrating genetic indicators into monitoring programs may improve evaluations of resilience and the long-term effectiveness of stock rebuilding under ICCAT management.

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Bi-decadal changes in the hydrozoan community of Otranto (SE Italy): responses to rising temperatures and tropicalization

Hydrozoans (Cnidaria) represents a key taxon of aquatic epibenthic communities. Their species composition and relative abundance can vary markedly across seasons in response to changing environmental conditions. As a primary driver of hydrozoan phenology and population dynamics, seawater temperature regulates the timing of developmental switches within their life cycles, thereby shaping seasonal shifts in community structure. Furthermore, owing to their high morphological plasticity, species richness, and ecological specialization, benthic hydroid polyps serve as reliable, persistent biological indicators of environmental change.

Within this framework, visually oriented collections of hydroids were carried out in June, August, October, and December 2024 along a vertical rocky wall near Otranto (SE Italy), spanning six bathymetric zones between depths of 0 and 30 m. Collected specimens were identified, and the resulting dataset was compared with surveys conducted at the same site and in the same periods in 2004, using the identical methodology.

Alongside a marked increase in mean seawater temperature – coinciding with one of the most severe marine heatwaves ever recorded in the Mediterranean Sea – a substantial decline in species diversity was observed, with the number of recorded species decreasing from 75 to 42. While many species documented in 2004 were no longer detected, there was a notable increase in the occurrence of both non-indigenous species (NIS) – such as *Eudendrium carneum* Clarke, 1882 and *E. merulum* Watson, 1985 – and native thermophilic species, such as the anthoathecate hydroid *Pennaria disticha* Goldfuss, 1820. This latter species, completely absent in 2004, emerged as the dominant hydroid taxon within the shallowest bathymetric zones across all sampling periods, with its distribution extending below 20 m depth in August.

These preliminary findings corroborate the prediction of *P. disticha* as a thermophilic invader and provide further evidence of the profound shifts currently altering Mediterranean benthic communities. Consequently, this study establishes a critical biodiversity baseline for future investigations on hydroid community responses to rising seawater temperatures and the increasing frequency and intensity of marine heatwaves.

Simposio 6

EVOLUZIONE MOLECOLARE, FILOGENESI E TASSONOMIA



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Appearances can be deceiving: Integrative taxonomy of the genus *Trapania* Pruvot-Fol, 1931 (Mollusca, Nudibranchia) in the Mediterranean Sea

In recent decades, increasing attention has been given to Mediterranean Nudibranchia molluscs, with a growing number of studies focusing on their high, and often overlooked, diversity. In this context, the genus *Trapania* was recently investigated and the systematics of the Northeastern Atlantic-Mediterranean species explored, leading to the introduction of important taxonomic changes, such as *T. hispalensis* Cervera & García-Gómez, 1989 and *T. pallida* Kress, 1968 being synonymized with *T. lineata* Haefelfinger, 1960. On the other hand, justified doubts have been raised about the validity of *T. fusca*, *T. tartanella* and *T. graeffei* due to their high anatomical similarity, in contrast to their distinct body colorations, which have been considered to date as the only diagnostic character able to distinguish them. Considering the scarcity of Mediterranean data available so far, we carried out an integrative taxonomic study on a more representative Mediterranean dataset, using optical and SEM microscopy, and a multilocus phylogeny based on Maximum Likelihood and Bayesian Inference, using two mitochondrial (16S and COI) and one nuclear (H3) molecular markers.

The results showed an even more complex scenario, with *T. pallida* reinstated as a valid species, being clearly distinct from *T. lineata*, and with *T. sanctipetrensis* Cervera et al., 2000 proposed as a junior synonym of *T. graeffei*. Regarding the latter species, it is noteworthy that molecular data, obtained from one specimen collected in the type locality, are provided here for the first time. Furthermore, the present study reports the first Mediterranean record of the South African *T. cirrita*, in different areas of the western and central Mediterranean basins. Finally, these results raise new questions on the possibility that the type species *T. fusca* (Lafont, 1874), may belong to the same '*T. graeffei* clade', given that the morphological differences used to define this species are primarily based on coloration, a character that has proven unreliable for delimiting species in this group. However, considering the lack of samples from the type locality, *T. fusca* remains provisionally valid.

This study highlights once again the importance of studying marine biodiversity through an integrated approach that simultaneously considers morphological, molecular and other complementary characters to obtain reliable results, ensuring taxonomic stability and accuracy, especially in Mediterranean marine invertebrates.

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The biodiversity of ascidians of the Venice Lagoon: taxonomic challenges from the integration of historical data, fieldwork, morphological and molecular analyses.

Biodiversity studies are crucial for environmental protection and species conservation, especially in vulnerable environments such as transitional waters, strongly threatened by human activities and biological invasions. The Venice Lagoon, the largest transitional system in the Mediterranean, exhibits pronounced spatial heterogeneity and is strongly influenced by human intervention and maritime traffic, making it prone to the introduction of new species. Moreover, high nutrient availability favors filter-feeding organisms, particularly ascidians, a key component of fouling communities and able to adapt to diverse environmental conditions. Their identification requires an integrative taxonomy approach, as they include numerous cryptic species that cannot be distinguished using morphology alone. The last comprehensive biodiversity study of ascidians in the Venice Lagoon dates to the 1970s, when 12 species were morphologically described. Although additional ascidian species have since been reported, no update has been conducted. In this work, we expanded knowledge of ascidian biodiversity in the Venice Lagoon by integrating literature data from the late 18th century onward, examining the evolution of nomenclature and its implications, and addressing challenges related to cryptic species, including the model species belonging to *Ciona* spp. and *Botryllus* spp. By using systematic review, we found that fewer than 20% of ascidians are currently identifiable using DNA barcoding, and less than 2% have a sequenced mitochondrial genome available for phylogenetic studies. These gaps indicate that public molecular databases remain insufficient to represent ascidian biodiversity reliably. Furthermore, we investigated the current ascidian biodiversity through two years of seasonal samplings, finding that eight historically reported species were not found, while 10 new records were identified among 23 species, including four non-indigenous species. The latter included also *Didemnum pseudovexillum* Turon & Viard 2020, the cryptic species of the highly invasive *Didemnum vexillum* Kott 2002, whose identification and impact assessment are particularly difficult. In conclusion, the lack of recent studies, together with species crypticity and the highly ascidian invasive potential, resulted in significant knowledge gaps regarding their biodiversity in the lagoon over the past 50 years, suggesting that continuous monitoring is necessary.

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Integrating phylogenomics and microRNAs resolves gastrotrich relationships within Spiralia

Deep relationships within Spiralia remain poorly resolved, especially for microscopic lineages such as Gastrotricha (Macrodasyida + Chaetonotida), historically underrepresented in phylogenomics. Here, we assembled one of the largest spiralian genomic datasets to date (75 species, 15 phyla), and inferred phylogenies from BUSCO genes from multiple matrices, differing in completeness, trimming, evolutionary model and recoding strategy.
Our analyses recover strong support for several major clades, including Lophotrochozoa, Chaetognathifera, and Kryptrochozoa among others. Mixture models consistently outperform partition models and amino acid recoding, indicating that information loss associated to recoding likely outweighs its benefit in correcting compositional bias. Rouphozoa (Gastrotricha + Platyhelminthes) is robustly monophyletic across nearly all analyses, whereas gastrotrich monophyly is sensitive to model complexity, gene number and taxon sampling, with simpler models grouping Chaetonotida with flatworms instead. A multivariate assessment of locus quality identified a gene subset fully supporting monophyly of both Rouphozoa and Gastrotricha, indicating that previously reported gastrotrich paraphyly reflects systematic bias rather than genuine signal.

Since gain and loss of microRNA families are rarely reversed over evolutionary time, we reconstructed the gastrotrich microRNA complement, currently absent from MirGeneDB, via new small RNA-seq libraries as an independent test. The resulting presence/absence patterns independently corroborate both rouphozoan and gastrotrich monophyly: the gastrotrich complement resembles that of flatworms, further supporting microRNAs as additional phylogenetic markers. Furthermore, we identify novel lineage-specific microRNAs: one diagnostic of Gastrotricha as a whole, and one restricted to Macrodasyida.

By combining denser taxon sampling, gene selection and model-independent genomic characters, our study confirms Rouphozoa, supports a single origin for Gastrotricha, and demonstrates the power of integrating phylogenomics and microRNA data to resolve recalcitrant phylogenetic relationships.

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A Curated DNA Barcode Library for Italian Wild Bees: from Monitoring to Integrative Taxonomy

Wild bees (Anthophila) are taxonomically diverse, ecologically pivotal pollinators, yet reliable molecular identification remains limited by incomplete and unevenly validated reference libraries. This gap is particularly critical in the Mediterranean region, where species richness, endemism, island biogeography and complex taxonomy converge. Here, we present a curated reference dataset for the COI barcode region of Italian Anthophila, designed to support taxonomy and biodiversity assessment.

More than 5,000 morphologically identified individuals, representing approximately 500 species, were examined. Although this sampling does not yet encompass the full diversity of Italian bees, further sampling is ongoing to expand taxonomic and geographic coverage. Morphological identifications were performed before sequencing the standard COI barcode region. Sequence data were quality-controlled, linked to voucher information and compared with public records to integrate newly generated barcodes with available data.

The dataset substantially increases COI coverage for Italian Anthophila and provides a geographically structured reference spanning mainland and insular bees. Preliminary analyses revealed patterns of genetic diversity at multiple taxonomic levels and highlighted potential taxonomic warnings. The dataset was also tested for identification performance across primer sets and COI regions, allowing evaluation of the shortest regions retaining species-level discriminatory power, something very useful for metabarcoding-based monitoring and when dealing with degraded DNA.

As a case study, we also identified a bumblebee group with multiple taxonomic warnings, the *Bombus flavidus* species group. The integration of the sequences generated in this framework, those obtained from specimens loaned from collaborators, and those retrieved from public repositories allowed to cover the entire distributional range of the target species and to test species hypotheses using an integrative approach. The integration of phylogenetics, DNA-based species delimitation, morphological data, and morphometric analyses revealed the presence of three additional species in the group. This example illustrates how curated barcode libraries can help highlight misidentifications, geographically structured lineages and cryptic or pseudo-cryptic diversity, and provide a stronger basis for reliable bee monitoring and conservation.

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Into the Unknown: Unravelling Genetic Diversity and Cryptic Lineages in the Mediterranean Rough Skate *Raja radula* Delaroche, 1809

In the last decades, several studies have been extensively investigated the species diversity of the genus *Raja* with the aim to resolve taxonomic boundaries and the potential underestimation of regional biodiversity. For this reason, integrative taxonomy application has become a fundamental approach for resolving species boundaries by combining molecular, morphological, and biogeographic evidence and resulting particularly useful in groups characterized by morphological conservatism or cryptic evolution as widely observed in the rajid species.

In the Mediterranean Sea, recognized as one of the world's major biodiversity hotspots, one of the rarest and least investigated species is the rough skate *Raja radula* Delaroche, 1809, despite its worrying state of conservation (Endangered in Global, Europe and Mediterranean IUCN Red List assessments). This species is distributed throughout the whole Mediterranean Sea and in the adjacent Atlantic Ocean waters of Portugal and Morocco. In spite of its wide distribution, knowledge concerning its population structure and intraspecific variability remains limited.

During the present study, we investigated the intraspecific diversity and population connectivity of the species through the analysis of mitochondrial markers (COI, NADH2 and 12S). To reach this scope, newly produced sequences were compared with those already available in online databases.

The obtained outcomes provide the first multi marker dataset for *Raja radula*, covering most of its geographical distribution at the Mediterranean scale, laying the basis for future research and conservation actions. In particular, our findings indicate high levels of genetic variability with signals of strong genetic population structure and low connectivity among the Mediterranean sub-regions. Moreover, our results suggest the possible occurrence of a cryptic lineage or species within the Mediterranean Sea; this specific topic requires urgent further investigation using multidisciplinary approaches.

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From Antarctica to the Arctic: dating the phylogeographic history of the tardigrade genus *Acutuncus*

Tardigrades are micrometazoans found worldwide. In Antarctica, they represent a major component of terrestrial meiofauna. The origin of Antarctic tardigrades is still debated and has been interpreted through two non-mutually exclusive hypotheses: the Glacial Refugia Hypothesis, suggesting that extant fauna derive from lineages already present on the continent before its progressive isolation and the onset of major glaciation during the Paleogene (66-23 Ma), and the Recolonization Hypothesis, suggesting that glaciation caused extinctions, and extant species recolonized the continent through dispersal events. *Acutuncus antarcticus* is the best-known Antarctic tardigrade species, being widely and densely distributed across the continent. For a long time, *Acutuncus* was believed to be monospecific and exclusively Antarctic. However, three additional species have recently been described from Europe (Italy and United Kingdom) and the Arctic, each showing a very restricted distribution. This pattern raises questions on the timing and processes that shaped the evolutionary history and current distribution of the genus. The aim of our study was to find out when the Antarctic lineage colonized or diversified in Antarctica through a molecular-clock framework. Whole Genome Amplification and Sequencing were performed on 45 individuals representing Antarctic, Arctic and European lineages. From these data, we conducted species delimitation and timetree inference with a set of mitochondrial and BUSCO protein-coding genes using a combination of concatenated and coalescent approaches while integrating the few fossil calibrations available for Tardigrada. Our results point out the presence of an undescribed cryptic species of *Acutuncus* in Antarctica, syntopic with *A. antarcticus* in a few regions. Our phylogeny supports the monophyly of *Acutuncus* and recovers two main lineages: an Antarctic lineage and an Arctic-European lineage. Molecular dating suggests that the split between these two lineages dates to the Late Cretaceous (100.5-66 Ma), during an advanced phase of the Gondwana breakup, whereas the divergence between Antarctic species is more recent, probably occurring during the Paleogene. These estimates suggest that extant species of *Acutuncus* are the outcome of an ancient lineage separation, reflecting geographic isolation, persistence of Antarctic populations and recent diversification events, pointing towards the glacial refugial scenario.

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A matter of peduncles: integrative systematics reshapes the genus *Aspidiophorus* (Gastrotricha, Chaetonotida)

The taxonomy of Gastrotricha is currently being reshaped by molecular phylogenetics, revealing widespread incongruence between morphology-based classifications and evolutionary relationships. Within Chaetonotidae, the most species-rich family of the phylum, diagnostic characters such as cuticular ornamentation have proved highly homoplastic, resulting in extensive para- and polyphyly of several established genera. The genus *Aspidiophorus*, traditionally defined by the presence of pedunculated scales, represents one of the clearest examples of this taxonomic instability. Here, we reassessed the evolutionary relationships within *Aspidiophorus* by substantially expanding its molecular sampling. Thirteen newly sequenced terminals were added to the dataset, including five marine (one new to science) and seven freshwater taxa (three new to science). Phylogenetic relationships were inferred using a concatenated multilocus dataset (18S rDNA, 28S rDNA and COI mtDNA) analysed within a broad Chaetonotida framework. Molecular evidence was integrated with detailed morphological investigations to provide a comprehensive systematic assessment. Our analyses recovered a deep and strongly supported split between marine and freshwater lineages. The marine clade was resolved as the earliest-diverging lineage within Chaetonotida, sister to all remaining taxa. This topology suggests that pedunculated scales may represent a plesiomorphic condition within Paucitubulatina. In contrast, freshwater representatives were distributed among at least three independent clades within Oiorpata, indicating multiple homoplastic origins of pedunculated scales in freshwater environments. Based on these findings, we propose the establishment of a new genus to accommodate the monophyletic marine lineage, supported by congruent morphological, molecular and ecological evidence. Our results provide a substantially improved phylogenetic framework for *Aspidiophorus*, support a deep marine origin of pedunculated scale-bearing gastrotrichs, and call for a comprehensive taxonomic revision of freshwater species currently assigned to this genus.

REBECCA FUNARI[1,2]

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Genomic footprints of an invader: reconstructing the origin, spread, and success of *Popillia japonica*

Invasive species pose an increasing global threat due to their ability to rapidly spread and adapt to novel environments, with significant ecological and economic consequences, highlighting the need to understand the mechanisms underlying invasion success.

The Japanese beetle, *Popillia japonica* Newman, native to Japan, is a major invasive pest feeding on hundreds of plant species, threatening agriculture and biodiversity in non-native regions including the U.S.A., Canada, the Azores, Italy, and Switzerland.

To support research into its ecology, evolution, and genetically informed control measures, we generated a high-quality, annotated genome for *P. japonica*. Building on this resource, we used whole-genome sequencing (WGS) and Specific locus amplified fragment sequencing (SLAF-seq) data to investigate population structure across the species' range. Global WGS data allowed the reconstruction of *P. japonica*'s invasion routes from Japan to the U.S.A. and Europe: analyses reveal an ancestral divergence between South and North/Central Japan, with the latter as the source of the initial invasion to U.S.A., while coalescent simulations support independent bridgehead events from the USA to the Azores and Italy. Focusing on the most recent movements in Europe, WGS data reveal distinct expansion/invasion patterns in Switzerland, where the pest was first detected in 2017. Populations in Basel, Valais, and central Switzerland likely originated from Ticino/Northern Italy via road or rail transport, while the Kloten population, near the Zürich airport, represents an independent introduction from North America, likely via air transport. As to the Azores, SLAF-seq data reveal the archipelago's fine-scale population structure, distinguishing a main cluster from two genetically isolated island populations.

Beyond reconstructing these pathways, WGS-based analyses suggest that strong selection is not a major driver of invasion success; instead, comparative analysis points to the genomic expansion of odorant receptor, ionotropic receptor, and cytochrome P450 gene families as pre-adaptive genomic features supporting the species' polyphagy and adaptability.

Together, these genomic and population-genetic datasets clarify the basis of *P. japonica*'s invasive potential and history, underscoring the need for continued monitoring, identification of invasion pathways, and development of biotechnologically oriented control strategies.

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Evolutionary consequences of host plant association in *cacopsylla melanoneura*: evidence from comparative genomics with co-generic species

Apple proliferation (AP) is a severe disease affecting European apple cultivation and is caused by the bacterial pathogen *'Candidatus Phytoplasma mali'* (*'Ca. P. mali'*). The primary insect vectors of *'Ca. P. mali'* are the apple psyllids *Cacopsylla melanoneura* and *C. picta*. *C. melanoneura* is associated with both apple and hawthorn trees and, in regions where *C. picta* is absent, serves as the sole vector responsible for the spread of *'Ca. P. mali'* in apple orchards. Laboratory studies suggest that populations developing on apple and hawthorn differ ecologically.

The aim of this study was to identify the genetic basis of host-associated reproductive preferences in *C. melanoneura* and to define evolutionarily significant units within the species. To achieve this, we generated a reference nuclear genome for *C. melanoneura* using a combination of long- and short-read sequencing technologies. We also sequenced genomes of closely related species within the same genus that exhibit similar reproductive host-plant associations. Using these newly assembled genomes as references, we investigated genetic variation associated with host preference in sympatric wild populations collected from apple and hawthorn trees across Europe, including Austria, Italy, Germany, the Czech Republic, Romania, and Serbia. Mitochondrial analyses indicate that *C. melanoneura* represents a species complex comprising at least two cryptic lineages, despite the absence of known morphological differences. Furthermore, nuclear SNPs analyses support the existence of two genetically distinct groups associated with different host preferences throughout Europe.

These findings provide valuable insights into the genetic basis of reproductive host preferences in *C. melanoneura* and will contribute to improved monitoring and management of *'Ca. P. mali'* vectors across Europe.

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The interactions between arthropods and the bacterial endosymbiont *Lariskella* (*Rickettsiales*) depend on a combination of bacterial phylogeny and host lifestyle

Arthropods engage in multifaceted interactions with a wide range of bacteria. Among the best studied cases are nutritional mutualists, providing their host with necessary compounds scarce in their diet, and reproductive manipulators. The bacterial roles are often variable depending on combinations of host and symbiont species and genotypes, external conditions, and subject to evolutionary variations. *Lariskella arthropodorum* (*Rickettsiales*, *Midichloriaceae*) is a host-dependent bacterium retrieved in association with a wide range of arthropods, particularly multiple insect orders and ticks. Despite a growing number of reports worldwide, in general the interactions of *Lariskella* with its host are poorly understood, with the exception of a recent study indicating that it can manipulate the reproductive of the stinkbug *Leptoglossus zonatus*.

Here we aimed to understand the functional features of the interaction between arthropods and *Lariskella* from an evolutionary viewpoint, particularly focusing on evolutionary transitions between different roles. For this purpose, we adopted a comparative genomics approach, sequencing novel *Lariskella* genomes from phylogenetically and ecologically diverse hosts, namely the tick *Ixodes persulcatus*, the flea *Xenopsylla cheopsis*, and the phytophagous beetle *Brassicogethes matronalis*. The bacterial genomes were selectively assembled from metagenomic sequencing reads of their respective hosts, and combined together with available *Lariskella* genomes from public repositories, as well as with representative relatives among *Rickettsiales*. This dataset was subjected to phylogenomic analyses and comparative genomic analyses, including pseudogene identification, metabolic and functional pathway reconstruction and comparison.

Accordingly, we identified three major subclades within *Lariskella*, which may constitute as many different species. The earliest diverging clade and one of the other two are typically associated with insects, the third one is tick-specific, indicating repeated host species shifts. While all *Lariskella* share many genomic traits, the tick-associated ones bear some traits indicative of recent genome reduction, as well as the potential to provide nutritional supplements to the haematophagous host diet. Accordingly, our analyses suggest that the interactions of *Lariskella* with its arthropod hosts are primarily shaped by the bacterial phylogeny, as well as partly by host eco-physiological features and lifestyle.

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Inter-kingdom horizontal gene transfer of carotenoid biosynthesis genes from fungi to the springtail *Bilobella aurantiaca*

Horizontal gene transfer (HGT) is increasingly recognized as a major driver of genome evolution, enabling the acquisition of novel metabolic capabilities. Among these, carotenoid biosynthesis represents one of the most remarkable examples of horizontal acquisition in only a few arthropod lineages. Here, we investigated the evolutionary origin and expression of carotenoid biosynthesis genes in the springtail *Bilobella aurantiaca*, a soil-dwelling species characterized by its distinctive yellow-orange pigmentation.

Genome annotation identified two adjacent carotenoid biosynthesis genes, *Phytoene synthase/Carotenoid cyclase* (*PS/CC*) and *Carotenoid desaturase* (*CD*), located on the same genomic scaffold and separated by less then 1 kb, consistent with a conserved syntenic organization. Comparative phylogenetic analyses indicate that both genes were acquired through an inter-kingdom HGT event from a fungal donor. To validate the genomic predictions, endpoint PCR and sequencing were performed on specimens from an Italian population, independently confirming the presence and primary sequence of both genes previously identified in a published genome assembly. Transcriptomic analyses demonstrated that both genes are transcribed, and their expression was further confirmed by quantitative real-time PCR (RT-qPCR).

The conserved genomic organization, experimental validation of gene presence, and consistent transcriptional evidence strongly support the stable integration and expression of the transferred genes in *B. aurantiaca*. Together with the characteristic body pigmentation of this species, these findings are consistent with the presence of a functional fungal-derived carotenoid biosynthetic pathway. Our results provide new evidence that inter-kingdom HGT contributed to metabolic innovation in Collembola and expand the growing number of animal lineages in which carotenoid biosynthesis has evolved through the acquisition of fungal genes.

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From genes to fiber: uncovering the molecular and biomechanical basis of silk production in the webspinner *Embia thyrrenica*

Human societies have long drawn on nature's resources, obtaining materials that have transformed cultures and economies. Among these, lepidopteran silk has become a cornerstone of the global textile industry, while spider silk has more recently attracted scientific attention for its remarkable mechanical properties, with applications spanning regenerative medicine, biomaterials development, and drug delivery. Yet silk-like materials have evolved independently across multiple animal lineages, most of which remain largely unexplored - representing an exciting avenue for the discovery of novel bioinspired materials. One such overlooked group is the webspinners (order Embioptera), insects that produce silk from specialized glands housed within the enlarged basitarsomeres of their forelegs. Despite their unique biology, the molecular mechanisms and evolutionary origins of embiopteran silk remain poorly understood.

We present an integrative, interdisciplinary study of silk production in *Embia thyrrenica*, a Mediterranean endemic. We established laboratory colonies of *E. thyrrenica* and generated a high-quality reference genome using long-read and long-range sequencing technologies. We then combined spatially resolved transcriptomic to identify the cellular and molecular machinery underlying fiber synthesis. To characterize gland morphology, we used scanning electron microscopy and X-ray nanotomography to reconstruct three-dimensional models of the basitarsomere silk glands. Finally, we coupled metabolomic and proteomic analyses with tensile testing to quantify the chemical composition and mechanical properties of the silk fibers.

By integrating molecular, cellular, and biomechanical scales, our analyses reveal how novel levels of biological organization emerge over evolutionary time. This framework not only illuminates the developmental and evolutionary origins of embiopteran silk but is also expected to establish design principles for next-generation bioinspired materials.

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Widespread Purifying Selection Overshadows ERC Signatures of Mitonuclear Coevolution at the Gene Level in Birds

Oxidative phosphorylation (OXPHOS) function is maintained by the synergistic interaction between nuclear and mitochondrial proteins. Given the pivotal role of OXPHOS in energy production in eukaryotes, coevolution between mitochondrial and nuclear genomes is expected and has been reported in many models. However, the precise molecular mechanisms shaping this coadaptation remain poorly understood across all eukaryotes. Investigating this evolutionary process is particularly compelling in birds, as their active flight and chromosomal sex determination create conditions where such coevolutionary pressures should be especially pronounced. Given the unique metabolic demands of avian flight, we anticipated a remarkably strong coevolutionary signal in birds, potentially exceeding that observed in other taxa. However, our analyses revealed unexpectedly weak signals despite this context. Using Evolutionary Rate Covariation (ERC) analyses on a dataset comprising 259 bird species, we found that mitochondrial genes do not exhibit significantly higher correlations with nuclear OXPHOS genes than with randomly selected genes. This result holds even when the examination was performed by grouping by complex or by a single gene. Nevertheless, a signal was recovered at the finest gene-level scale, proving that mitochondrial-contacting nuclear subunits show the expected higher correlation than non-contacting ones. Along with this, we detected generally low rates of synonymous and nonsynonymous substitution consistent with widespread purifying selection acting across the avian genome, which could explain the weak ERC results. While these findings do not entirely rule out mitonuclear coevolution, they indicate that, in birds, the process is neither detectable via ERC nor manifested at the gene level.

Simposio 7

DIVULGAZIONE, DIDATTICA E MUSEOLOGIA



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The museum heritage of the University of Bari: beyond conservation

The University of Bari is home to thirteen museums covering the humanities, the life sciences, medicine, the natural, chemical, and physical sciences. These comprise a variety of different museum types and collections, ranging from collections of historical scientific instruments (Chemistry; Physics) to traditional (Zoology, Hearth Sciences) and "mixed" museums (Botanical Garden Museum and Herbarium).

This heritage results from research activities or has been accumulated through donations or purchases. Its origins, in many cases, date back to the establishment of the first faculties, thus representing important historical and cultural testimony that has spanned over the long history; therefore, museums are the natural repository of artefacts that have played a decisive role in the advancement of scientific research.

Today, the museum heritage of the University of Bari has acquired extraordinary value in supporting teaching, education, and scientific dissemination, constituting a true bridge between science and the local community. University museums, indeed, represent a channel of communication with the local community, providing support for the knowledge, use, and protection of the territory itself.

The museums of the University of Bari, as well as many other Italian universities, are now organized into a University Museum System (SiMA), which, as a central organizational structure, assumes the task of protecting and enhancing the heritage they contain, according to a coherent and shared logic and in accordance with national and international regulations and guidelines.

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From Invisible Biodiversity to Public Engagement: An Inclusive Model for Zoological Outreach

Despite its fundamental ecological role, much of animal biodiversity remains invisible to the general public, limiting awareness of its importance for ecosystem functioning and conservation. To address this gap, the University of Urbino Carlo Bo developed the D.O.V.E. (Diversity – Observation – Value – Education) project that combines zoological research, education and public engagement into an inclusive learning experience centred on biodiversity.

The project brings together interactive laboratories, direct observation of terrestrial and marine organisms, photographic exhibitions, and gamified learning activities within a single educational pathway. Rather than presenting zoology as a collection of isolated facts, the initiative encourages participants to discover biodiversity through observation, exploration and dialogue, promoting scientific curiosity and critical thinking.

The educational activities were designed according to inclusive teaching principles, allowing the active participation of heterogeneous audiences, including students with special educational needs and neurodivergent learners. The initiative was developed in collaboration with Azobè ODV, Archivio Loreno Sguanci and Washington Patrignani Museum. By combining visual, practical and interactive experiences, the initiative offers multiple access points to scientific knowledge while fostering awareness of the role of often-overlooked organisms.

Beyond educational outcomes, the initiative strengthens the connection between university research and society, demonstrating how zoological knowledge can be communicated through complementary approaches that integrate scientific accuracy, experiential learning and visual dissemination. In this perspective, photographic exhibitions and zoological materials become not only educational resources but also cultural tools capable of stimulating reflection on biodiversity conservation and the relationship between humans and nature.

This experience proposes a replicable model for zoological outreach that integrates research, education and public engagement, highlighting the role of biodiversity as a shared natural heritage and reinforcing the contribution of zoological sciences to scientific literacy, environmental responsibility and social inclusion.

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The photography exhibition “A changing Mediterranean Sea”: a case of scientific dissemination and awareness-raising

Opened in May 2026, the photography exhibition "A changing Mediterranean Sea. The Climate Change in the Salento Seas" is exposed, from May to June and for the Winter Season, at the Museum of Environment (MAUS) at the Ecotekne campus, in Lecce, and, during the Summer season, at the Marine Biology Museum “Pietro Parenzan” in Porto Cesareo (LE), two Museums of the University of Salento Museal System. These Museums are the best option to reach the more students and the more tourists possible considering their strategic positions into the University campus and in a touristic locality such as Porto Cesareo, ensuring an effective transfer of knowledge from the scientific community to society. This is also a ‘traveling exhibition’, designed to be easily moved, bringing the topic of climate change out of the classroom and closer to the community. Through 24 shots made by the nature photographer Michele Solca, the exhibition highlights the ongoing changes affecting the Salento Seas by documenting, with scientific rigor and visual power, the effects of global warming on a geographic area considered a hot-spot of Mediterranean marine biodiversity. The exhibition develops its visual narrative around four main themes i) rising sea temperatures and their effects on certain organisms ii) the changing balance between native and Non-Native Species (NIS), iii) the possible key drivers of NIS spread, and iv) the possible future conservation strategies based on scientific research, sustainable fisheries and the increasing awareness of individual citizens. The fundamental role of the zoology in understanding the biodiversity and of the museums as prisms for disseminating ideas and concepts, is emphasized by two panels entirely dedicated to the scientific research conducted by the different laboratories of the Department of Biological and Environmental Sciences and Technologies (DiSTeBA) of the University of Salento. Furthermore, showing beautiful photographs of our amazing sea at a time of rapid change, promotes a contrasting feeling of appreciation and wonder, and awareness of what we are losing, essential to reach the main goal of a University Scientific Museum. In fact, through these powerful feelings, the photographic exhibition and the hosting Museums, can combine knowledge of biodiversity with good practices that reflect and convey greater civic responsibility towards the marine environment.

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The dioramas of the Natural History Museum of Milano, a successful exhibition tool for over 60 years

The Natural History Museum of Milan is the most ancient civic museum of the city and has a long tradition of scientific dissemination since 1838. The zoological exhibitions have drastically changed over the last sixty years from a classic systematic criterion to a more modern ecological key showing animals in their environments and emphasizing the close link between organisms and their habitat. The tool that was chosen in the ’60 was the diorama, a representation of natural scenarios containing mounted specimens arranged in in a forecast that recreates their native environment, including soil, vegetation, rocks and water. The first attempts were 16 small dioramas about Italian fauna made under the management of Edgardo Moltoni in the early 1950s, but they were later dismantled. The current older diorama in the Museum represents the green anaconda in the Brazilian Amazon and was made in 1965. In 1968 the former director Cesare Conci presented a plan for the renewal of the exhibition based on dioramas that was completed on 2017, mainly thanks to the effort of Luigi Cagnolaro. The Museum exhibition now comprises 97 dioramas (two paleontological, 11 entomological and 84 for vertebrate zoology), one of the most numerous of the world. The vertebrate zoology exhibition is organized to represent the main biomes of the world in nine halls and the Italian fauna in three more halls.

Many showcases illustrating the ecology and behavior of many species living in the environments showed in the halls flank the dioramas. To date, no multimedia was used in our exhibitions, that contain only mounted specimens or models. This was a precise choice dictated by conceptual and practical motivations: first, the curators are convinced that visitors come to the museum to come into contact with real specimens giving them an immersive experience and information about animal real appearance and size. A plethora of videos and interactive media is available on the web and visitors do not really need to visit a museum to see them. Second, multimedia is expensive and need continuous updating and maintenance to keep up with technological improvement and obsolescence and this could raise funding problems for a public institution. Furthermore, interactive devices create bottlenecks in the visitor flow and this could be a problem in a museum visited by more than 250,000 persons every year. Dioramas proved to be a useful tool for educational purposes even after many years.

Tavola Rotonda

DIGITALIZZAZIONE DELLA BIODIVERSITÀ, WHERE NOW, WHAT'S NEXT?

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ItaBOL - the Italian Barcode of Life Initiative

The Italian Barcode of Life (ItaBOL) is the national node of a broader international community advancing DNA barcoding and DNA metabarcoding as powerful tools for biodiversity research, assessment, monitoring, and management. ItaBOL is conceived as an open, inclusive, and interdisciplinary community bringing together scientists and stakeholders interested in applying these approaches across a wide range of biological disciplines and applications. The initiative aims to strengthen collaboration at the national level while maintaining strong connections with the International Barcode of Life (iBOL) and iBOL Europe. ItaBOL will promote best practices across fieldwork, laboratory procedures, and bioinformatics, including shared infrastructures, coordinated sampling campaigns, and the development of reference DNA sequence libraries for flora, fauna and funga across Italy and the broader Mediterranean region, linked to well-documented specimens and associated metadata. These efforts will support integrative taxonomy studies and biodiversity assessment at local and global scales, across terrestrial and aquatic ecosystems, targeting species of ecological, agricultural, and conservation relevance, while also enabling applications in food security, forensic science, and the monitoring and detection of pest and allochthonous species. Currently, 30 institutions are involved in the ItaBOL network, mainly universities and research organizations, together with a natural history museum and a cultural organization, with more than 110 researchers across Italy having signed the Memorandum of Understanding. This diversity reflects ItaBOL's commitment to inclusivity, interdisciplinarity, gender balance, and diverse expertise and career stages. To organize and coordinate its activities, ItaBOL has been structured around a scientific council and six working groups, which are currently starting their activities. Beyond research, ItaBOL will disseminate knowledge and foster the uptake of DNA-based identification tools among stakeholders, including policymakers, environmental agencies, and industry. By integrating scientific excellence with collaborative governance and open participation, including public engagement, ItaBOL aims to become a national reference point, in synergy with other major national biodiversity, and bioinformatics initiatives, and a key contributor to global initiatives in the field.

Sessione poster

EVO-DEVO:

DALLA MORFOGENESI ALL'EPIGENOMICA



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Looking back: unravelling embryonic development of two iconic Mediterranean nudibranchs (Mollusca, Gastropoda)

Nudibranchs are specialized gastropods, with extraordinary defensive strategies evolved in parallel with the loss of the shell in adults. They are simultaneous hermaphrodites and oviparous, with gelatinous egg ribbons laid on different substrates. They show a biphasic life cycle, with two different larvae and both direct and indirect development. The first larval stage (trochophore) develops in the egg while the second (veliger) may undergo either indirect development, being planktotrophic or lecithotrophic, or directly metamorphosize into the egg, hatching as juvenile. These data highlight a great developmental variability, with species switching from indirect to direct and from lecithotrophic to planktotrophic depending on environmental conditions. The current knowledge on nudibranch's embryonic development and metamorphosis, from the zygote to the mature veliger and/or post-metamorphic stage, is scanty and mainly limited to few single extra-Mediterranean cases. Considering the importance of the Mediterranean Sea as a hot spot of diversity and aiming to fill gaps in the knowledge of its fauna, we have documented the development, under controlled laboratory conditions, of the two Mediterranean iconic *Cratena peregrina* (Gmelin, 1791) and *Peltodoris atromaculata* Bergh, 1880. After spawning, egg masses were monitored daily, and cleavage, morula, blastula, gastrula, trochophore, veliger and juvenile photographed through optical microscopy. Both species exhibited indirect development with free-swimming mature veliger, but with differences in timing, morphology and behaviour. Veliger of *C. peregrina* has a small shell, reduced velar region, well-visible statocysts and an intense swimming activity. Furthermore, the development was markedly rapid (ca. 8 days). In contrast, *P. atromaculata* veliger shows a bigger shell, widely ciliated velum, well-defined foot, propodium and larval eyes, and a reduced movement. The development was much slower (ca. 21 days). Additionally, after 30 days, *P. atromaculata* veliger settled and metamorphosizes progressively losing the velum and the shell. These results suggest *C. peregrina* is planktotrophic with prolonged dispersal, while *P. atromaculata* is lecithotrophic with early metamorphosis. This is the first detailed report of embryonic development and metamorphosis of Mediterranean species, becoming a fundamental baseline for future studies on embryogenesis and climate-change-related survival of Mediterranean nudibranchs.

POSTER N° 1

Sessione poster

ZOOLOGIA AL CONFINE: MULTI, INTER, TRANS-DISCIPLINARITÀ



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Nanoparticle-mediated CRISPR/Cas9 delivery in *Hermetia illucens* for enzymatic PET degradation

Hermetia illucens is increasingly recognized as a valuable organism for the bioconversion of organic waste and the development of sustainable biotechnological processes. Expanding its metabolic capabilities through genetic engineering could further enhance its potential in environmental applications, including the biodegradation of recalcitrant pollutants such as synthetic plastics.

In this study, we investigated the feasibility of delivering gene-editing components to *H. illucens* using biodegradable chitosan-based nanocarriers. Nanocapsules were designed for the encapsulation of CRISPR/Cas9 systems aimed at enabling the expression of polyethylene terephthalate (PET)-degrading enzymes in insect tissues. Nanoparticles were produced through ionic gelation and water-in-oil emulsification approaches and characterized in terms of size distribution, morphology, colloidal stability, and release behaviour.

Particular attention was devoted to the biological compatibility of the formulations. *In vitro* toxicity assays are currently being performed using Drosophila-derived cell lines as an insect cellular model to identify concentration ranges compatible with cell viability and to determine the minimum toxic concentration of the nanocarriers. In parallel, preliminary *in vivo* experiments in *H. illucens* larvae have been initiated to evaluate nanoparticle administration, tolerance, and potential uptake under physiological conditions.

Ongoing studies will assess delivery efficiency through fluorescence-based analyses and molecular approaches for the detection of genome-editing events. These investigations will provide essential information on the suitability of chitosan nanocarriers for genetic manipulation of this insect and on the feasibility of exploiting this species as a platform for the expression of heterologous enzymes involved in plastic degradation.

By integrating insect physiology, nanotechnology, and molecular biotechnology, this work contributes to the development of innovative strategies aimed at enhancing the environmental and biotechnological value of *H. illucens*.

POSTER N° 2

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Exploring the biological role of polyhydroxynaphthoquinones in *Paracentrotus lividus*: tissue distribution, antioxidant activity, and seasonal dynamics

Sea urchins (Echinoidea, Echinodermata) comprise over 800 living species worldwide. Among them, *Paracentrotus lividus* is one of the main edible Mediterranean species. Sea urchins are the only known producers of polyhydroxynaphthoquinones (PHNQs), or spinochromes, secondary metabolites responsible for their pigmentation. PHNQs exhibit antioxidant, antimicrobial, anti-inflammatory, cardioprotective, anticancer, and antidiabetic activities, although their physiological role remains poorly understood. This study investigated the distribution, abundance, antioxidant activity, and seasonal variation of PHNQs in *P. lividus* across tests, spines, intestines, and coelomic fluid, and their relationship with seawater temperature and solar irradiation.

Monthly samplings were performed at Le Grazie (La Spezia, Italy) from May 2024 to June 2025. Thirty individuals were collected each month, and selected tissues were dissected, lyophilized, and extracted. PHNQs were identified and quantified by UHPLC-ESI-TOF-HRMS. Five compounds were analysed: Spinochrome A (SpA), Spinochrome B (SpB), Spinochrome E (SpE), Spinochrome 282 (Sp282), and Echinochrome A (EchA). Antioxidant activity was evaluated using the ABTS assay (EC50).

PHNQs showed tissue-specific distributions. Intestines yielded the highest PHNQ extraction, although lipid content and digested food complicated the analyses. Spines showed the highest antioxidant activity, followed by tests, supporting a photoprotective role against UV-induced oxidative stress. SpE and SpB were mainly detected in skeletal tissues and were absent from coelomic fluid, suggesting a role in external defence. SpA and Sp282 were detected in all compartments, with the highest concentrations in intestines. EchA was also most abundant in intestines but absent from coelomic fluid. The accumulation of Sp282 in intestines suggests a role in protection against food-associated microorganisms.

Overall PHNQ production and antioxidant activity showed no clear seasonal pattern and were not significantly correlated with seawater temperature or solar irradiation. However, monthly variations of SpE, EchA, and SpA in skeletal tissues appeared consistent with the reproductive cycle of P. lividus. These findings improve our understanding of PHNQ physiological roles and support sea urchin by-products as a sustainable source of bioactive compounds.

POSTER N° 3

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Vitellogenesis characterization and peptide-based immunoassay development for vitellogenin detection in the Norway lobster *Nephrops norvegicus*

Vitellogenin (VTG) is the major yolk precursor protein required for normal oocyte growth and maturation. It is widely recognized as a valuable biomarker of reproductive status and endocrine disruption in marine organisms. Among them, the Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758) (Homarida: Decapoda), is one of the most valuable decapod crustaceans exploited throughout the European continental shelf and slope.

The present study aimed to characterize its ovarian and embryonic development and to develop an indirect enzyme-linked immunosorbent assay (ELISA) for VTG detection to assess its usefulness in investigating its reproductive cycle. Females were assigned to one of six reproductive developmental stages based on ovarian morphology. Ovaries and, when present, externally carried eggs were collected for histological analyses. Oocyte developmental stages were identified, quantified, and measured in histological sections, while fecundity was estimated in ovigerous females by determining the total number of eggs attached to the pleopods. Samples of hepatopancreas, ovaries, and external eggs were also collected for VTG analysis. A conserved VTG peptide was identified through comparative sequence analysis of decapod crustaceans, chemically synthesized, and used to produce polyclonal antibodies. These antibodies were subsequently employed to develop an indirect ELISA, which was validated using hepatopancreas, ovarian, and egg extracts obtained from females at different reproductive stages. The generated polyclonal antibodies specifically recognized VTG in *N. norvegicus*, enabling the first ELISA-based quantification of this protein in the species. The immunoassay detected significant differences in VTG abundance among hepatopancreas, ovaries, and eggs, revealing tissue-specific and seasonal expression patterns that closely reflected the reproductive cycle.

Overall, this study provides the first comprehensive characterization of VTG distribution in *N. norvegicus* and introduces a reliable immunological tool for monitoring reproductive physiology. The developed ELISA has considerable potential for applications in reproductive biology, ecotoxicological studies, and environmental biomonitoring of marine decapod crustaceans.

POSTER N° 4

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Morpho-functional principles of *Paracentrotus lividus* pedicellariae for interdisciplinary biomimetic design

Sea urchin pedicellariae are specialized stalked appendages (150 µm-3 mm) displaying remarkable morphological and functional diversity. In *Paracentrotus lividus*, ophicephalous, globiferous, tridentate and trifoliate pedicellariae differ in valve morphology, articulation and motility, reflecting their functional specialization like grasping, defence and self-cleanign.

This study investigated their morpho-functional organization to identify transferable biological principles through an interdisciplinary biomimetic workflow integrating Zoology and Design. Pedicellariae were analysed using scanning electron microscopy (SEM) and stereomicroscopy to characterize skeletal architecture, valve articulation and movement patterns. Moreover, in vivo video recordings were acquired to characterize pedicellariae movements and their responses to external stimuli. The analyses highlighted several morpho-functional features with high biomimetic potential, including tripartite grasping, staggered valve articulation and pressure-driven opening, enhanced by serrated valve margins for secure grip. These features were interpreted as constructional principles rather than directly replicated, allowing their functional abstraction and transfer into design. The interdisciplinary study was based on bidirectional knowledge transfer, with zoologists identifying biological functions and designers translating them into functional solutions. Design requirements stimulated targeted biological investigations, whereas zoological findings progressively refined the design concept, generating an iterative biomimetic workflow and a shared methodological language. The selected principles were integrated into the development of a bioinspired plogging stick inspired by ophicephalous pedicellariae. The three-valve gripping mechanism provides a selective, stable and non-slip grasp on litter of different shapes, while the serrated texture, derived from the natural valve morphology, improves both object retention and handle ergonomics during outdoor activities. Rather than replicating the biological model, the final product translates its most effective functional strategies into an innovative tool that promotes litter collection and contributes to pollution reduction, demonstrating how zoological research can contribute to sustainable solutions through interdisciplinary biomimetics.

POSTER N° 5

ALICE PUGNO[1], FRANCESCA MINGOIA[1], GIOVANNI BOCCHI[1], RENATO BACCHETTA[1], FRANCESCO BONASORO[1], CARLA LANGELLA[2], ALESSANDRA MICHELETTI[1], SARA IGNOTO[1], MICHELA SUGNI[1]

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The madreporite of *Paracentrotus lividus*: a model for interdisciplinary biomimetics through bidirectional knowledge transfer

Paracentrotus lividus represents a valuable model for biomimetic research owing to its distinctive morpho-functional adaptations. Among these, the madreporite plays a key role in regulating seawater flow within the water vascular system. This study aimed to characterize its morpho-functional organization and to develop a biology-driven design process based on a close integration between Zoology and Design.

The madreporite was investigated using scanning electron microscopy (SEM), histological analyses, micro-computed tomography (micro-CT) coupled with three-dimensional modelling, and in vivo functional assays. In particular, live specimens were exposed to fluorescent NanoPET particles to visualize the filtration process in vivo, highlighting the contribution of the ciliated epithelium in addition to the skeletal canal system. The analyses highlighted a marked functional regionalization of the canal network, confirming the madreporite as a highly specialized system for filtration and fluid-flow regulation. The study is founded on bidirectional knowledge transfer between Zoology and Design. Rather than a one-way transfer of biological knowledge to design, it evolved through an iterative collaboration in which zoologists and designers developed a shared language and continuously guided each other's research. Design challenges stimulated new biological investigations, while zoological findings guided design decisions, resulting in an integrated biomimetic workflow.

Within this collaborative framework, a Life Cycle Assessment (LCA) was also performed to evaluate the environmental impact of the proposed product throughout its entire life cycle. In design, LCA is a fundamental tool for eco-design and circular economy, supporting the selection of solutions with the lowest environmental impact. Its integration into this project also strengthens the zoological perspective by promoting nature-inspired solutions that contribute to environmental sustainability and biodiversity conservation. The interdisciplinary workflow led to the development of a bioinspired fruit bowl in which a network of microchannels, inspired by the madreporite, directs ripening gases toward a zeolite-containing compartment, slowing fruit maturation. This study demonstrates how zoological research can drive sustainable innovation through biomimetics, establishing an effective framework for interdisciplinary collaboration and bidirectional knowledge transfer between biology and design.

POSTER N° 6

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DNA metabarcoding reveals overlooked freshwater Gastrotricha diversity

Freshwater Gastrotricha are among the least documented components of the freshwater meiofauna, and their diversity is likely substantially underestimated. We evaluated their diversity in Italian inland waters using DNA metabarcoding and compared the molecular results with taxonomic identifications based on light microscopy.

Twelve freshwater lentic biotopes distributed across four Italian regions were surveyed. From each biotope, bulk sediment samples were collected for taxonomic identification by light microscopy, while sediment cores (upper 5-10 cm) were collected for DNA metabarcoding. Meiofaunal organisms were separated from the sediment by Ludox density-gradient centrifugation, recovered by filtration on a 32-µm mesh sieve, and processed for DNA extraction. Two mini-barcode markers targeting the V4 region of the *18S rRNA* gene and a portion of the Folmer mtCOI fragment were amplified and sequenced using Illumina MiSeq paired-end technology. Amplicon sequence variants (ASVs) were generated in QIIME2 and taxonomically assigned using marker-specific bioinformatic pipelines and curated reference databases. DNA metabarcoding recovered gastrotrich ASVs from every surveyed biotope, including five in which no Gastrotricha were detected by light microscopy and one dry pond, demonstrating its ability to reveal diversity overlooked by morphological analyses. The 18S and COI datasets yielded 401 and 327 gastrotrich ASVs, respectively. Taxonomic resolution improved substantially through the use of curated reference databases, allowing more reliable assignments while highlighting current gaps in molecular reference data for freshwater Gastrotricha.

Overall, DNA metabarcoding proved to be a powerful tool for assessing freshwater gastrotrich diversity, substantially expanding biodiversity detection compared with light microscopy alone. Our findings demonstrate the value of integrating molecular and morphological approaches to document overlooked components of freshwater meiofaunal communities and highlight the need to further expand curated molecular reference databases for Gastrotricha.

POSTER N° 7

Sessione poster

ADATTAMENTI FENOTIPICI ED INTERAZIONI: DALLA CELLULA AL COMPORTAMENTO



GIULIA FIORENZA, MARTINA PAVESI, GIULIANO GASPERI, ALBERTA PINNOLA, MARIA VITTORIA MANCINI, ANNA R. MALACRIDA, FEDERICO FORNERIS, RICCARDO PICCINNO

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WICKED – *Wolbachia* Infection: Corrupted Killers Effects on *Drosophila suzukii*

Drosophila suzukii is an invasive pest from South-Eastern Asia that has successfully colonized most temperate regions worldwide. Given its unique ability to lay eggs into ripening fruits, in addition to be highly polyphagous, this species poses a significant threat to agriculture. Every year, *D. suzukii* causes severe damage to numerous crop species, particularly soft fruits such as cherries, strawberries, and blueberries, leading to substantial crop losses and subsequent economic damage. Currently, control strategies are challenging and rely mainly on non-target, environmentally harmful insecticides. The endosymbiont *Wolbachia* represents a promising tool for the biocontrol of this fly. However, *wSuz*, the native strain of the bacteria harboured by *D. suzukii*, does not induce Cytoplasmic Incompatibility (CI) and thus do not present any negative impact on the reproductive success. In this study, we investigated a population of *D. suzukii* in Sondrio, Italy. We evaluated the prevalence of *wSuz* within the population, and we will elucidate potential fitness advantages through laboratory fitness, fecundity and fertility assays, particularly associated with overwintering survival, accompanied by differential gene expression analysis. The results showed seasonal variations in its prevalence, with a higher occurrence during warmer months. Although significant differences were observed across the distinct sampling sites, these require further investigation to rule out potential bias, given that their statistical significance was borderline. Conversely, both temperature and atmospheric pressure were found to impact prevalence dynamics: specifically, *Wolbachia* prevalence increased with rising temperatures and decreasing atmospheric pressure. The second objective of this study regards the mechanisms underlying this lack of CI, to unveil the crucial structural residues necessary for such process. To achieve it, we are currently employing structural biology techniques on recombinant CI factors (CifA and CifB) to obtain their structure and characterize their interaction. Elucidating these structures will guide the development of an effective, species specific, and eco-friendly control strategy leveraging *wSuz*. Preliminary *in-silico* predictions suggest that, despite the high sequence similarity with the CI-inducing *w*Mel strain (harboured in *D. melanogaster*), there are significant structural differences in the interaction dynamics between these CI factors.

POSTER N° 8

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Light-Dark Behavioral Responses to Different Wavelengths in the Branchiopod *Lepidurus couesii*

Light is an important environmental cue that influences the behavior and ecology of many aquatic invertebrates. Phototactic responses can affect habitat selection, predator avoidance, foraging activity, and environmental exploration, making light a key factor shaping interactions between organisms and their environment. Responses to light have been documented in several crustaceans, including Branchiopods. *Triops granarius* exhibits negative phototaxis, and recent studies have demonstrated wavelength-dependent behavioral responses in branchiopods under different spectral conditions. These findings suggest that light quality may play a significant role in shaping behavioral decisions in branchiopods. However, little is known about how these responses vary across wavelengths in Notostracan. Therefore, the present study aims to investigate the wavelength-dependent light-dark behavioral responses in *Lepidurus couesii*.

Trials were conducted under controlled laboratory conditions using a two-compartment apparatus consisting of an illuminated and a dark zone. A total of 70 individuals were exposed to four wavelengths of light (white, green, blue and red), and the time spent in the illuminated compartment (TSL) was recorded during 10-minute trials. Differences among wavelengths were evaluated using a non-parametric Friedman ANOVA followed by Durbin-Conover post hoc comparisons.

Across all light conditions, individuals spent a greater proportion of the trial duration in the dark compartment than in the illuminated compartment. Behavioral responses differed significantly among wavelengths (Friedman ANOVA, $p < 0.001$). Individuals spent the greatest amount of time in illuminated areas under red light (3.70 min) and the least under blue light (0.94 min), while green (1.85 min) and white (1.63 min) exhibited intermediate responses. Durbin-Conover post hoc comparisons further showed that responses under red light differed significantly from all white, green and blue conditions.

Overall, the observed behavioral patterns suggest that *Lepidurus couesii* exhibits negative phototaxis, as individuals spent most of the trial duration in the dark compartment. However, the observed differences among wavelengths indicate that the strength of this behavioral response varies under different light conditions. These findings establish a baseline for future studies examining the ecological significance of light in Notostraca.

POSTER N° 9

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Unexpected and remarkable record of *Herpyllobius* sp. (Copepoda, Herpyllobiidae) parasitic from Mediterranean polynoid polychaete *Harmothoe antilopes*

Parasitic lifestyles within the copepod family Herpyllobiidae lead to extreme phenotypic adaptations, including a complete loss of segmentation and appendages in adult females alongside male microsomia. In fact, in the hyper-diverse genus *Herpyllobius*, the female body comprises two portions connected by a short stalk: an internal endosoma, which anchors the parasite and absorbs nutrients from the host, and an external ectosoma bearing two genital swellings with ovisacs. Although these highly modified mesoparasites are well-documented on polynoid polychaetes across temperate and polar latitudes (0-350 m depth), their occurrence in the Mediterranean Sea remains enigmatic and scarcely reported, representing a significant gap in our understanding of this exclusive relationship.

Here, we report an unexpected and remarkable record of a mature female *Herpyllobius* sp. collected at a depth of 45 m off the coast of Salivoli Marina (Livorno Province, Tyrrhenian Sea) during the *ante-operam* environmental monitoring campaigns for the SACOI III project. The host was identified as the polynoid polychaete *Harmothoe antilopes*. Preserved in 85% ethanol, the parasite specimen exhibits a unique combination of morphological features, including a smooth, whitish integument, a short stalk, a pear-shaped ectosoma with a single dorsal hump, without a lateral furrowed surface, symmetrical genital swellings possessing elongated ovisacs with rounded edges and an elongated cylindrical endosoma, without digitiform processes. These features show remarkable differences from *Herpyllobius arcticus*, the only herpyllobiid currently known in Italian waters, suggesting that this is a new species, although the specific identification is in progress.

In conclusion, this finding significantly expands the recorded geographical range of the genus *Herpyllobius* and it increases our knowledge of this specific association, which is little documented in the Mediterranean basin. This work supports advanced morphological and molecular investigations to identify a potential new species within the Italian benthic biodiversity.

POSTER N° 10

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Brain organization in Ground Beetles: a synchrotron-based comparative study

Coleoptera is the most species-rich and one of the most extensively studied insect orders, yet its nervous system remains strikingly understudied at the neuroanatomical level. Quantitative, comparative brain data are scarce for beetles overall and virtually absent for carabid beetles, limited to a few classical histological descriptions. This gap persists despite new powerful technologies are now available for tackling insect neurosciences. We present here a comparative neuroanatomical study of five carabid species, selected to represent different ecological specialization within the family, from obligate social parasitism to free-living predation and phytophagy: *Paussus favieri* representing one of the most specialized ant nest parasites, *Brachinus sclopeta* a nocturnal generalist predator and scavenger, *Asaphidion curtum* and *Notiophilus rufipes*, litter predators and *Amara aenea*, a diurnal spermophagus. Brain reconstructions rely exclusively on synchrotron-based X-ray micro-computed tomography, combining phase-contrast and absorption modalities to achieve high-resolution, non-destructive neuropil segmentation without any histological sectioning, an approach still largely unexplored in insect comparative neuroanatomy. Through 3D reconstruction and analysis software we quantified major brain neuropils, which are morphologically distinct association areas of the brain, across all species within a single, standardised imaging pipeline. Our work is thus framed in a comparative neurobiological context, exploring how ecological specialisations shape sensory investment and behavioural flexibility across the family. A further contribution of this work lies in refining synchrotron-based imaging protocols for neural tissue, comparing different contrast-staining approaches and techniques to identify the optimal strategy for visualising fine soft-tissue details. This constitutes the first multi-species, synchrotron-based neuroanatomical comparison for ground beetles, and one of the very few for Coleoptera as a whole, providing a novel quantitative baseline for how ecological specialisation reshapes brain organisation within the most diverse insect order on Earth.

POSTER N° 11

Sessione poster

IMPATTI AMBIENTALI E STRATEGIE DI CONSERVAZIONE DELLA BIODIVERSITÀ



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Assessment of breeding site status and *Batrachochytrium dendrobatidis* screening in *Bombina variegata pachypus* populations from Molise

The endemic Apennine yellow-bellied toad *Bombina variegata pachypus* is considered one of the most threatened Italian amphibians. It is classified as Endangered in the Italian IUCN Vertebrate Red List and listed in Annexes II and IV of the Habitats Directive. Over the last 30 years, *B*. *v*. *pachypus* appears to have undergone a generalized population decline throughout its distributional range, with an estimated loss of at least half of its historical distribution records. The main threats include the degradation and loss of breeding habitats and the emergence of chytridiomycosis, an infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), which is considered one of the major factors underlying global amphibian declines.

In Molise, *B*. *v*. *pachypus* is currently distributed mainly in mountain areas, while it has apparently disappeared from lowland and hilly areas where it was historically reported. Current knowledge of the conservation status of regional populations is limited and, to the best of our knowledge, pathogen screening to test for the presence of Bd in *B*. *v*. *pachypus* populations has never been performed in the region.

In this study, we assess the status of breeding sites across most known Molise populations and conduct the first regional Bd screening through skin swabbing of adult individuals, followed by qPCR assays for Bd detection. Preliminary results indicate that several breeding sites have disappeared, while others have undergone a reduction in habitat suitability for reproduction, highlighting the need for urgent conservation actions aimed at maintaining or restoring suitable breeding habitats. Although no sampled individuals showed evident clinical signs compatible with chytridiomycosis during field surveys, Bd screening will provide the first evidence on the possible occurrence of the pathogen in Molise populations.

POSTER N° 12

ALESSANDRO BORSOTTI[1,2], SARA BORTOLINI[1,3], MARTINA FALAGIARDA[2], BEATRICE DELAITI[2], MANFRED WOLF[2], HANNES SCHULER[1,3]

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Species-specific ddPCR detection of unhatched oophagous parasitoids in stink bug egg masses

The brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), is a highly polyphagous invasive species responsible for severe agricultural losses worldwide. To limit its spread, several countries have implemented biological control programs based on the release of the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae), the primary egg parasitoid of *H. halys* in its native range. Its introduction requires long-term monitoring to assess its effects on native and invasive stink bugs, and to evaluate its competitive interactions with native parasitoid species. However, such impacts may remain undetected due to the phenomenon known as “egg abortion” or an “evolutionary trap”, in which parasitoids fail to complete development in novel hosts lacking a shared co-evolutionary history. This outcome is particularly common in environments where native and non-native species interact and ultimately results in killing of the host egg without leaving visible signs of parasitization.

Here, we present a highly sensitive digital droplet PCR (ddPCR) assay designed to detect hidden parasitization events in unhatched stink bug egg masses from three native and non-native species. The assay employs species-specific primers and probes targeting eight parasitoid species commonly associated with stink bug egg masses and will be capable of detecting both single and combined multiparasitism events. The analysis will be applied to field-collected egg masses originating from an area undergoing a biological control program and comparisons will be performed across egg masses sampled in consecutive years to capture temporal changes in host–parasitoid interactions. The resulting data are expected to uncover previously unrecognized trophic relationships and provide a high-resolution tool for monitoring potential non-target effects within a biological control program.

POSTER N° 13

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eDNA metabarcoding of soil microarthropods: how biochar shapes community composition in Italian vineyards

Biochar has emerged as a Nature-Based solution to improve crop productivity and soil quality in agricultural systems. While numerous studies have highlighted its positive effects on soil structure and physicochemical properties, relatively little attention has been paid to its impact on soil faunal communities. Soil microarthropods play a crucial role in ecosystem functioning, contributing to nutrient cycling, organic matter decomposition, and overall soil health. Previous studies investigating the effects of biochar on soil fauna have mainly focused on ecotoxicological aspects, whereas community-level responses under real field conditions remain largely unexplored.

The present study addresses this knowledge gap by evaluating the effects of biochar amendment on soil microarthropod communities through an environmental DNA (eDNA) metabarcoding approach. Soil samples were collected from three organic vineyards located in the Chianti Classico region of Tuscany, Italy. The vineyards were amended with biochar at a rate of 15 t ha^{-1} in 2023 and differed in soil characteristics while being managed under the same viticultural practices.

A total of 24 soil samples were collected from each vineyard — 12 from control and 12 from biochar-treated parcels. The mitochondrial marker COI will be amplified, and sequencing data will be taxonomically assigned to family, genus and species level (depending on marker resolution and reference database coverage), enabling a comprehensive characterization of soil microarthropod communities.

We hypothesize that long-term biochar amendment promotes changes in soil microarthropod community composition and increases taxonomic diversity by improving soil habitat quality. This study will provide novel insights into the long-term effects of biochar on soil biodiversity in Mediterranean vineyards and highlight the potential of eDNA metabarcoding as a powerful tool for soil fauna monitoring.

POSTER N° 14

LAURA MAGARIELLI[1,2], LORENZO GAUDIANO[2], GAETANO DI LISO[1], FRANCESCA VENTURA[1], FABIO QUINTO[3], GIANLUCA CIRELLI[3], BIAGIO SAMPOGNA[4], GIUSEPPE CORRIERO[1]

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Assessing mammal occurrence and trapping rates within coastal riverine SACs of Basilicata (southern Italy)

Estuarine environments and river mouths are critical ecosystems characterized by high biodiversity but also by marked vulnerability to anthropogenic pressures, including the spread of invasive alien species. This study characterizes the medium- and large-sized mammal community within three Natura 2000 Special Areas of Conservation (SAC) along the Ionian-Metapontino coast: "Costa ionica foce Basento" (IT9220085), "Costa ionica foce Cavone" (IT9220095), and "Costa ionica foce Agri" (IT9220080). Monitoring was conducted using a standardized camera-trapping design, with 9 survey stations distributed across each river system. Two consecutive sampling sessions were carried out in temporal continuity: spring 2025 and autumn 2025. Each session consisted of a standardized effort of 40 consecutive days per station. To avoid overestimation and double-counting, independent photographic events were defined using a one-hour threshold for each species, allowing the calculation of species-specific trapping rates as indices of relative abundance.

The sampling activity detected 9 species, revealing a complex community where native carnivores of high conservation priority, specifically the Eurasian otter (*Lutra lutra*) and the wolf (*Canis lupus*), coexist with generalist mesocarnivores such as the red fox (*Vulpes vulpes*), European badger (*Meles meles*), and stone marten (*Martes foina*). The assemblage also includes ecologically impactful species: the native wild boar (*Sus scrofa*) and the invasive coypu (*Myocastor coypus*). As a semi-aquatic rodent of Union concern, *M. coypus* poses a major ecological threat and is currently subject to regional eradication plans under Legislative Decree 230/2017. Inter-seasonal comparison showed marked fluctuations in trapping rates and spatial distribution. In spring, apex predators (wolf and otter) were restricted exclusively to SAC IT9220080, expanding their range to include SAC IT9220085 in autumn. Conversely, the invasive coypu exhibited strict habitat fidelity, occurring solely within SAC IT9220095 across both seasons.

The obtained results update the ecological baseline of these estuarine ecosystems, confirm their high conservation value, and provide key information to support management actions, particularly for ecologically impactful species such as the wild boar and the coypu.

POSTER N° 15

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Migrants from warm seas: an example with Serpulidae (Annelida)

The Mediterranean marine fauna is undergoing significant changes due to the introduction of non-indigenous species, mainly originating from tropical regions and facilitated by global warming. For several years, the Zoology Laboratory at the University of Salento has been conducting faunal and taxonomic research on various groups of marine invertebrates, particularly polychaete annelids, mollusks, crustaceans, and cnidarians. These studies have highlighted the need for further taxonomic and biogeographical investigations on both Mediterranean and Indo-Pacific species to better understand patterns of biodiversity and invasion pathways.

Here, we analyzed the distribution of species belonging to the family Serpulidae (Annelida), which includes numerous non-indigenous taxa, with particular focus on the genera *Hydroides*, *Spirobranchus*, and *Serpula*. These sessile, calcareous tube-building polychaetes are common components of marine fouling communities colonizing ship hulls, which represent one of the main vectors for their dispersal and introduction into new areas. Although the presence of sibling species may require molecular analyses for reliable identification, most serpulids can be readily distinguished based on morphological characters, particularly the operculum, a specialized structure that seals the opening of the calcareous tube.

The genus *Hydroides* is represented in the Mediterranean by 17 species, 12 of which are non-indigenous. *Spirobranchus* includes six Mediterranean species, two of which are non-native, while *Serpula* comprises six species, including one non-indigenous taxon. Consequently, more than 50% of the species belonging to these three genera in the Mediterranean are introduced. This study examines their distributions along the Mediterranean basin and the Italian coasts, relating their occurrence to their geographic origin and invasion pathways.

Most records of non-indigenous species are currently concentrated in the eastern Mediterranean and many have not yet reached the Italian coasts. Among the introduction pathways, Lessepsian migration through the Suez Canal appears to be one of the most important drivers shaping the current distribution of these species.

POSTER N° 16

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Microplastic Interference with Calcium Carbonate Biomineralization: An Integrated Abiotic and Biotic Approach

Microplastics (MPs) are increasingly recognized as potential disruptors of marine calcification, yet their effects on biomineralization remain poorly resolved because particle–mineral interactions and organism-mediated processes are generally investigated separately. Here, we present an integrated experimental framework to assess how MPs influence calcium carbonate formation under both abiotic and biotic conditions, using the sea urchin skeleton and calcifying symbiotic bacteria as complementary model systems.

In the abiotic system, isolated sea urchin skeletal ossicles are used as biologically structured calcitic substrates during controlled $CaCO_3$ precipitation in the presence of MPs. This approach tests whether particles modify heterogeneous nucleation, crystal growth, polymorph selection, surface coverage and incorporation within newly formed mineral phases. In parallel, symbiotic bacterial isolates capable of inducing $CaCO_3$ precipitation are exposed to MPs to determine whether the particles affect bacterial growth, mineralization efficiency, crystal morphology and mineral–cell associations.

The resulting precipitates are characterized through scanning electron microscopy and energy-dispersive X-ray spectroscopy to evaluate morphology and elemental distribution, together with Raman spectroscopy and X-ray diffraction for mineral-phase identification. Preliminary observations indicate that MPs can modify the spatial distribution and morphology of $CaCO_3$ crystals, although the magnitude and direction of the response depend on particle physicochemical properties and on whether mineralization occurs through an abiotic or biologically mediated pathway.

By directly comparing these systems, this study distinguishes interfacial effects occurring at the particle–mineral boundary from indirect effects mediated by skeletal surface and microbial activity. The work provides a clearer understanding of how MPs may interfere with calcification in marine invertebrates and their associated microbiota, while establishing the sea urchin skeleton as a relevant model for investigating pollutant-driven alterations of biomineralization across biological scales.

POSTER N° 17

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Small-scale fisheries in two MPAs: monitoring challenges and implications for biodiversity conservation

Marine Protected Areas (MPAs) are key tools for marine biodiversity conservation, promoting habitat protection and sustainable resource use. However, fishing activities continue within many MPAs, making effective management and co-management measures essential. The design and evaluation of these measures depend on reliable information on fishing effort and catches. This study assessed the quality of fishery logbook data by comparing self-reported records with independent onboard and landing monitoring data collected in the *Penisola del Sinis–Isola di Mal di Ventre* and *Capo Carbonara* MPAs.

In the Sinis MPA, 86 fishing trips recorded between 2018 and 2023 were available in both datasets. Paired Wilcoxon tests revealed significant differences in reported catches for trammel nets ($V = 413.5$, $p = 0.004$) and traps ($V = 123$, $p = 0.002$), whereas no significant differences were detected for fishing effort. Bland–Altman analyses highlighted opposite reporting biases, with fishers overestimating trammel-net catches (bias = +6.22 kg) and underestimating trap catches (bias = −7.53 kg). In addition, about 30% of monitored fishing trips were absent from logbooks. In the Capo Carbonara MPA, discrepancies were even greater, with 82% of monitored activities lacking corresponding records, preventing robust statistical analyses.

Results indicate that the main limitation of the current monitoring system is not only reporting accuracy but, above all, data incompleteness. The omission of a substantial proportion of fishing activities may lead to underestimation of fishing pressure and catches, affecting the evaluation of management effectiveness within MPAs. These findings highlight the need for improved data collection and validation through participatory monitoring and adaptive co-management approaches. At the same time, they raise questions about the actual capacity to assess conservation measures in MPAs characterized by relatively large fishing fleets, such as the Sinis MPA, where uncertainty in fisheries data may limit the reliability of management evaluations.

POSTER N° 18

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Unexpected rapid *ex-situ* growth in the psammobiotic sponge *Oceanapia isodictyiformis* (Porifera, Demospongiae)

Endopsammic sponges exhibit remarkable adaptations to survive buried within marine sediments, playing a fundamental role in substrate consolidation and oxygenation. Despite their ecological importance, their growth dynamics and biological mechanisms remain largely underexplored.

This study provides the first morphometric observation and *ex-situ* growth monitoring of the Mediterranean psammobiotic sponge *Oceanapia isodictyiformis*. This species is characterized by an irregular basal mass that incorporates the surrounding sediment, from which numerous tubular fistules emerge vertically into the water column. Specimens were sampled from the shallow inlet of La Strea (Porto Cesareo, Ionian Sea), maintained in a controlled aquarium and photographed at three time points for a total of 26 days. Digital image processing (ImageJ) was utilized to monitor morphological variations (new fistules, branch development) and quantify growth. To investigate the skeletal dynamics associated with growth, the spicular sets of the fistules, branches, and basal parts were analyzed using optical microscopy.

During the 26-day experimental period, all specimens showed a positive and continuous growth trend. Total surface area increased in each individual: four specimens grew by 27–42%, while one showed an exceptional +255% increase (3.24 to 11.51 cm²). Notably, while no specimens possessed branches at the beginning of the experiment, most specimens developed new branches by the end. The fastest-growing specimen generated up to seven distinct branches alongside an increased number of primary fistules. Microscopic analysis revealed a heterogeneous distribution of spicules: while apical fistules were dominated by mature oxeas, the base and branches contained numerous thinner, incompletely silicified oxeas. Based on this structural evidence, it is hypothesized that the sediment-embedded basal portion functions as the generative zone for skeletogenesis, where newly formed spicules are actively produced and subsequently transported upward through the mesohyl to supply the actively growing branches. The observed branching, a trait occasionally found in natural habitats, is consistent with this rapid development and is a biological trait of the species. These findings highlight the remarkable morphological plasticity of *O. isodictyiformis* and establish a foundation for future investigations into the skeletal and cellular dynamics of psammobiotic sponges.

POSTER N° 19

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Distribution of *Pinctada radiata* in the Mar Piccolo of Taranto along a confinement gradient

In recent years the spread of Lessepsian species in Mediterranean transitional waters has raised growing concern, making it necessary to investigate the factors shaping their establishment and their interactions with native assemblages. Within this framework, the pearl oyster *Pinctada radiata* (Leach, 1814) (Bivalvia, Margaritidae), recorded in the Mediterranean since 1874, but recently responsible for marked demographic explosions in lagoon environments, was studied in the Mar Piccolo of Taranto (north-western Ionian Sea), a semi-enclosed transitional basin whose inner inlet is highly confined. The aim was to assess the distribution of *P. radiata* and the variation of population parameters in areas with different degree of confinement.

In September 2025 field sampling was carried out at about one metre depth across three stations and three hard-substrate types (stony bottom, vertical stone walls and mussel-farming poles). For each substrate, all individuals were collected within standard surfaces and their density and shell height measured. Moreover, the associated macrobenthic community was qualitatively and quantitatively characterised.

As observed in other Mediterranean lagoons, our results show that density of *P. radiata* decreased significantly along the confinement gradient, without significant differences in bivalve density among substrates. A well-structured population occurred in the two less confined stations, while the dominance of juveniles in the innermost station suggests strong selection by extreme hydrological variability. Notably, the rich associated benthic community, including several alien species, appeared unaffected by the presence of *P. radiata* even where the bivalve reached high densities.

POSTER N° 20

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Glacier springtails as sentinels of cryosphere biodiversity

Glacier ecosystems host a unique and still largely overlooked biodiversity that is rapidly threatened by climate-driven ice loss. Recent studies have revealed that glacier animals are far more diverse than previously assumed, while highlighting major knowledge gaps in their distribution, ecology, and conservation. Within this fauna, glacier springtails (Collembola) represent an exceptional model system. As highly specialized inhabitants of ice-associated habitats, they combine ecological sensitivity, probably limited dispersal ability with respect other widespread ice-dwelling invertebrate (i.e. tardigrades and rotifers), and strong associations with glacial environments, making them valuable indicators of cryosphere integrity.

Here, we present an overview of ongoing efforts to investigate glacier biodiversity through an integrated framework combining intensive ecological surveys, integrative taxonomy approaches, and citizen-science initiatives. Intensive sampling campaigns on Alpine model glaciers and large-scale samplings at global scale have uncovered the ecology and biodiversity of this understudied taxon. At the same time, the CollembolICE citizen-science network is expanding the spatial coverage of observations and collections, fostering public engagement in cryosphere research.

By linking ecological monitoring with phylogeographic analyses, glacier springtails provide a powerful tool to reconstruct past glacial dynamics, identify potential refugia, and assess the biological consequences of glacier retreat. We propose that these organisms can serve as effective sentinels for monitoring biodiversity change and guiding conservation strategies in rapidly disappearing glacier ecosystems.

POSTER N° 21

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Niche and spatial segregation define the distribution of sympatric red wood ant species

Interspecific competition is a key ecological and evolutionary mechanism shaping the spatial structure of communities. Species may reduce competition by differentiating their climatic or environmental niche and/or by limiting local co-occurrence. These mechanisms are expected to be particularly pronounced among closely related species, which typically share similar ecological requirements. An example of evolutionarily recent species is the *Formica rufa* species - group (red wood ants), which includes highly competitive species. Their overall distribution patterns suggest spatial segregation, yet comparative analyses across multiple species at a fine spatial scale remain scarce. Using a regional-scale approach encompassing seven co-occurring wood ant species (although two species resulted too rare to be included in all comparative analyses), we assessed patterns of niche differentiation and local co-occurrence. Our results showed a general niche equivalency among species, although the degree of niche overlap varied among species pairs. Lower overlap values were observed for *Formica rufa, F. polyctena*, and *F. pratensis*, whereas *F. lugubris* and *F. aquilonia* occupied more similar environmental spaces. The entire species group showed a strong association with coniferous trees and relatively low temperatures. Syntopy (local co-occurrence within 100 m) was rare among five sympatric species. Our results suggest that ecologically similar species can coexist regionally despite substantial niche overlap, while exhibiting limited local co-occurrence. By integrating niche and spatial segregation analyses, this study highlights contrasting patterns of coexistence among closely related red wood ant species.

POSTER N° 22

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Global priorities for vertebrate roadkill risk mitigation

Roadkills from wildlife-vehicle collisions represent a severe threat for wildlife, as they can endanger the persistence of local populations. While interest towards the topic of roadkill has increased, literature shows strong geographical and taxonomical biases, and no global analysis of roadkill drivers and patterns exists that encompasses all vertebrate groups and at high grid-resolution. To overcome these limitations, we used citizen science data to: 1) identify the drivers of roadkill risk for four classes of vertebrates (amphibians, reptiles, birds, and mammals), 2) produce global maps of roadkill risk at high grid-resolution (1 km^2), and 3) identify areas with high biodiversity and high roadkill risk (mitigation priorities). Data were gathered from the GBIF platform, selecting all records originating from the iNaturalist database (ResearchGrade observations only) with a positional uncertainty lower than 1 km. We tested 18 variables describing climate, land cover, and socio-economic factors. After collating data, we modelled (separately for each class) the probability of roadkill occurrence using the Maximum entropy algorithm. Cells with roadkills were considered as “presence” and 10000 background points were picked randomly, but proportionally to the sampling effort. We used a four-fold spatial block cross-validation to assess model transferability. Models were then projected to produce the first high-resolution global roadkill risk maps for terrestrial vertebrate classes, which were later overlaid with overall and threatened species richness maps provided by the IUCN to identify global hotspots of roadkill-related extinction risk at 10 km^2 resolution. Our results will aid to determine whether “universal” mitigation measures are sufficient or if effective conservation requires taxon-specific strategies. These products will set a new standard for biodiversity modelling in fragmented landscapes, facilitating the integration of opportunistic data into formal conservation planning. The project aims to build a road-resilient biodiversity network where infrastructure development and wildlife conservation are no longer in conflict, but co-managed through data-driven strategies.

POSTER N° 23

Sessione poster

EVOLUZIONE MOLECOLARE, FILOGENESI E TASSONOMIA



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Two Stings, One Sea: Linking genetics and morphology to reveal Sardinian stingrays' population structure and conservation priorities

In recent years, *Dasyatis tortonesei* Capapé, 1975 records have become increasingly frequent, and its geographical distribution range is continuously updated. Despite it, the species still suffers from biological, ecological, and fishery-related data shortages, hampering adequate conservation actions. In addition, data collection is challenging due to the taxonomic ambiguity that characterise *Dasyatis tortonesei* and *Dasyatis pastinaca* (Linnaeus, 1758), boosted by the morphological features shared by these two sympatric species.

With the present work, we aim to highlight morphological differences useful for the correct discrimination of the two species and, simultaneously, produce new and crucial data for the study and conservation of the common and Tortonese's stingrays in Sardinian waters. In particular, our purpose aims to fully describe the Sardinian populations from both genetic and demographic point of view, potentially identifying ecologically important areas for the studied species.

To reach our goal, specimens were identified using molecular tools, divided into two separated groups and then morphologically analysed. Performing molecular, together with both linear and geometric morphological analyses we obtained evidence of genetic and somatic discriminant features.

Our results highlight important juvenile's aggregation areas for both species along the Sardinian coasts. Additionally, molecular data indicates moderate to high levels of genetic diversity for the Sardinian stingrays' populations. The sums of these findings suggest that the studied area could represent a strategic site for the future conservation and protection of Sardinian population of both stingray species, *Dasyatis tortonesei* and *Dasyatis pastinaca*.

POSTER N° 24

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Hidden diversity in marine Gastrotricha: Mediterranean lineages are genetically distinct from North Sea species

Marine meiofaunal organisms present a long-standing biogeographic paradox: despite their limited dispersal ability, many nominal species have been reported to exhibit remarkably broad, even cosmopolitan, distributions. This pattern is particularly evident in Gastrotricha, where cryptic diversity and persistent taxonomic impediments have long obscured species boundaries. Here, we investigated whether four marine gastrotrich species shared between the North Sea and the Mediterranean Sea represent single widespread species or geographically structured evolutionary lineages. Populations of *Xenotrichula intermedia*, *Halichaetonotus aculifer*, *H. atlanticus*, and *Turbanella bocqueti* were analysed using Oxford Nanopore long-read sequencing, from which the nuclear (18S, 28S) and mitochondrial (COI) markers were recovered for multigene phylogenetic reconstruction. Species boundaries were subsequently evaluated using six complementary population genetic, distance-based, coalescent-based, and tree-based species-delimitation approaches (4× rule, ABGD, ASAP, GMYC, PTP and bPTP). All four nominal species consistently recovered Mediterranean and North Sea populations as distinct evolutionary lineages, although the strength of genetic divergence varied among taxa. *X. intermedia* exhibited the strongest pattern, with Mediterranean specimens further subdivided into distinct Ligurian, Adriatic and Tunisian lineages, revealing substantial cryptic diversity within the Mediterranean basin. Additional internal structuring was detected within the northern populations of *H. aculifer* and *T. bocqueti*, whereas *H. atlanticus* exhibited a shallower, although still consistent, north–south differentiation. Despite relying on conceptually different analytical frameworks, all species-delimitation methods converged on the same general pattern of lineage diversification, supporting the long-term evolutionary independence of the identified lineages. Overall, our results reveal a pervasive north–south phylogeographic structure and extensive hidden diversity across the investigated species. The recurrent recovery of geographically structured lineages in taxonomically and biologically diverse gastrotrich species suggests that repeated north–south phylogeographic divergence is a major driver of diversification in European marine Gastrotricha. This, in turn, indicates that hidden diversity is a widespread feature of marine Gastrotricha rather than the result of isolated cases of cryptic speciation.

POSTER N° 25

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Integrative taxonomy of European *Isotomurus* (Collembola: Isotomidae): preliminary evidence for cryptic diversity and taxonomic inconsistencies

The genus *Isotomurus* Börner, 1903 (Collembola: Isotomidae) is a taxonomically challenging group within springtails. Traditionally, species identification has been based on morphological traits, such as pigmentation pattern and chaetotaxy, characters which are often subject to a great deal of intraspecific variability. Previous morphological studies (Carapelli et al. 2001) have offered useful taxonomic frameworks and preliminary phylogenetic hypotheses based on detailed morphological analyses, but a comprehensive evaluation integrating both molecular and morphological evidence is still missing. The present study aims to explore species diversity and phylogenetic relationships among European *Isotomurus* using an integrative taxonomic approach. Specimens were collected in several localities of Italy and other areas of southwestern Europe, including taxa of systematic interest and endemic lineages. Preliminary species identification was based on traditional morphological characters in the taxonomy of the genus. Subsequently DNA was extracted to amplify the mitochondrial gene cytochrome c oxidase subunit I (*cox1*) to proceed with DNA barcoding analysis. Molecular and morphological data were integrated to assess species boundaries within *Isotomurus*. Preliminary phylogenetic analyses performed using *cox1* revealed two clearly separated and well-supported clades among the specimens identified as *I. ghibellinus* from Tuscany and France, suggesting the presence of previously unrecognized taxonomic lineages. Conversely, *I. aetnensis*, *I. pseudopalustris* and *I. maculatus* from Sicily clustered in the same clade despite currently being recognized as distinct species. These results suggest the existence of cryptic diversity and taxonomic inconsistencies in the genus. Ongoing work is focused on expanding the *cox1* dataset and establishing a curated reference library within the Barcode of Life Data System (BOLD). In parallel, whole-genome sequencing (WGS), as well as morphological analyses, are being performed to provide a phylogenomic framework for testing species boundaries and reconstructing evolutionary relationships within the group.

POSTER N° 26

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A Whole New World of Diversity: Unlocking Cape Verdean shark diversity with DNA barcoding, sport fishing and citizen science

Effective fisheries management and conservation planning depend on accurate species identification, particularly for taxa vulnerable to direct exploitation or incidental capture. Mitochondrial DNA barcoding offers a reliable tool for species identification, even among morphologically similar or cryptic taxa. The Republic of Cabo Verde hosts a remarkably diverse shark assemblage with over 50 species recorded, making it a crucial area for the conservation of global chondrichthyan diversity. Unfortunately, two-third of the Republic of Cabo Verde shark species is threatened, particularly by intense industrial fishing combined with poor management measures and climate change. Moreover, the area was scarcely investigated during the years, and important gaps in knowledge of local shark populations still remain. For this reason, accurate species identification is essential for developing effective conservation and management strategies.

In the present study, we applied a DNA barcoding approach to identify shark specimens caught on Boa Vista (Cape Verde) during a sport fishing trip by using mitochondrial DNA markers. In particular, we produced new molecular data for 4 putative species present in the area morphologically identified as *Carcharhinus brevipinna* (Müller & Henle, 1839), *Carcharhinus limbatus* (Valenciennes, 1839), *Paragaleus pectoralis* (Garman, 1906), and *Rhizoprionodon acutus* (Rüppell, 1837). Furthermore, tissue samples were collected from fins and jaws retrieved in local shops and fish markets and then analysed to identify them at the species level. According to preliminary morphological identification, fins and jaws belong to at least seven different genera (*Carcharhinus*, *Galeocerdo*, *Isurus*, *Negaprion*, *Prionace*, *Rhizoprionodon*, *Sphyrna*) recorded in the area.

This study enlarges the molecular data available on Cape Verdean sharks by providing new sequences for endangered species and their local populations, many of which have little genetic data available for the Cape Verdean archipelago.

POSTER N° 27

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ItaBOL - the Italian Barcode of Life Initiative: the national node advancing DNA-based biodiversity research and monitoring

The Italian Barcode of Life (ItaBOL) is the newly established national node of the International Barcode of Life community (iBOL) and iBOL Europe, created to coordinate and strengthen DNA barcoding and metabarcoding activities in Italy. ItaBOL provides an open and collaborative framework for researchers, institutions, museums, taxonomists, geneticists, bioinformaticians, ecologists, and stakeholders interested in the development and application of DNA-based tools for biodiversity research, monitoring, and management. A major goal of ItaBOL is to improve the availability, quality, and accessibility of reference DNA barcode data for Italian and Mediterranean biodiversity To this end, ItaBOL has started activities aimed at preparing reference datasets to be shared with the scientific community and at identifying current gaps in existing data resources, including BOLD. These actions will help define priorities for future sampling, sequencing, curation, and data integration. As a newly launched community, ItaBOL already brings together a large and highly motivated community of participant from across Italy and has organized its activities into six working groups, which are initiating actions on key topics including reference data, gap analyses, stakeholder engagement, dissemination, citizen science, and the harmonization of wet-lab protocols, metabarcoding approaches, and bioinformatic pipelines. ItaBOL works in connection with national initiatives and infrastructures such as the National Biodiversity Future Center (NBFC) and ELIXIR, strengthening synergies among biodiversity research, molecular data generation, bioinformatics, and data sharing. The study of Italian genetic diversity requires effort among taxonomists, geneticists, museum curators, bioinformaticians, ecologists, and specialists across organismal groups. ItaBOL invites all interested members of the scientific community to participate and contributi by sharing expertise, data, protocols, collections, infrastructures, and perspectives. By fostering coordinated action and open participation, ItaBOL aims to become a national reference point for DNA-based biodiversity research and a key contributor to European and global initiatives in the field.

POSTER N° 28

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New records of Symphyla (Myriapoda) from agricultural landscapes of Emilia-Romagna and Lombardy (Italy)

Symphylans (Myriapoda: Symphyla) are among the least studied soil arthropods, and their diversity, distribution, and ecology remain poorly known worldwide. Despite their ecological importance in soil ecosystems and their potential role in agricultural environments, faunistic and taxonomic data on Symphyla are still scarce in Italy, with only 7 genera and 19 species recorded from the country.

To improve the current knowledge of this neglected group, a preliminary survey was conducted in agricultural fields and gardens across the Emilia-Romagna and Lombardy regions (northern Italy), for a total of 6 sites in the provinces of Parma, Bologna, Ravenna, Cervia and Mantova. Soil and compost samples were collected from different sites and examined for the presence of symphylans.

The genus *Symphylella* was by far the most frequently recorded taxon. In particular, *Symphylella vulgaris* Hansen, 1903 was the dominant species and was found in a wide range of habitats, raising suspects that it may represent a complex of cryptic species. Individuals belonging to the genus *Scolopendrellopsis* were less abundant but consistently present across the surveyed sites. Remarkably, the collected material included a previously undescribed species of *Scolopendrellopsis*, which is currently under formal taxonomic description. Representatives of the genus *Scutigerella* were also recorded, although only a few specimens were collected, indicating a comparatively lower abundance in the investigated environments.

These findings provide new faunistic data on the symphylan fauna of northern Italy and contribute to the understanding of the distribution of Italian Symphyla. The discovery of an undescribed species highlights the still incomplete knowledge of this group and emphasizes the need for further taxonomic, faunistic, and ecological studies. Expanded surveys and detailed morphological and molecular investigations will be essential to clarify species diversity and distribution patterns of Symphyla in Italy and throughout Europe.

POSTER N° 29

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Diversity and distribution of freshwater copepods in Armenia

Freshwater copepods are key components of aquatic food webs and effective indicators of ecosystem structure and environmental change. However, global biodiversity shortfalls, such as the Linnean and Wallacean ones, hamper our knowledge of species identities and spatial distributions in many regions. Armenia exemplifies these gaps due to limited taxonomic surveys and sparse occurrence data. As a recognized biodiversity hotspot within the Caucasian ecoregion, Armenia hosts many endemics, yet its inland water zooplankton fauna remains poorly studied. Between 2025 and 2026 we surveyed about 150 sites across Armenia's elevational gradient, including permanent lakes, reservoirs, and temporary lakes and ponds, using 100 µm mesh plankton nets. Taxonomic identifications followed current keys and literature and were supported by DNA barcoding of representative specimens using the COI mtDNA marker.

We recorded a diverse copepod fauna comprising about 26 taxa across calanoid, cyclopoid and harpacticoid orders, with notable contributions from the genera *Arctodiaptomus* and *Cyclops*. Richness varied markedly among habitats, with endemics and subendemics taxa mostly limited to temporary ponds and high-altitude lakes, whereas invasive species are limited to low-altitude artificial water bodies. Several taxa represent new regional records or range extensions for the South Caucasus, including at least 8 species not previously documented in Armenia, and some taxa putatively new to Science. Calanoids community composition was strongly structured by altitude and water mineralization, indicating pronounced environmental filtering and potential vulnerability to anthropogenic pressures. whereas such a pattern was not observed for cyclopoids and harpacticoids. Moreover, species accumulation curves assessed with multiple asymptotic richness estimators applied to occurrence data indicate that actual copepod diversity in the region is substantially higher than observed, implying that many species likely remained undetected despite our intensive sampling effort. This work provides the first comprehensive assessment of freshwater copepod diversity across Armenian inland waters, aiming to address existing knowledge gaps and to support future biodiversity assessments. The discovery of previously unreported taxa highlights the need of continued taxonomic and molecular investigations to resolve cryptic diversity and establish robust baselines for monitoring and conservation effort.

POSTER N° 30

Sessione poster

DIVULGAZIONE, DIDATTICA E MUSEOLOGIA



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Citizen science reveals bait-fishing behaviour in the Yellow-legged Gull (*Larus michahellis*)

Bait fishing is a rare foraging strategy in which floating objects are used to attract aquatic prey. This behaviour has been documented in only a handful of species, particularly herons and egrets, and its apparent rarity makes it difficult to detect through conventional field studies. In this context, citizen science offers a valuable opportunity to identify and characterize unusual and innovative behaviours that might otherwise remain unnoticed.

Seagull GO! is a citizen science initiative specifically designed to investigate behavioural innovation in gulls. The project engages the public through pop-inspired graphics and video game references, encouraging participants to record and share observations of unusual gull behaviours. By combining scientific research with accessible communication, Seagull GO! aims to expand the opportunities for detecting rare behavioural events in these highly adaptable birds.

Through this project, we were able to analyse an opportunistic observation suggesting that wild yellow-legged gulls (*Larus michahellis*) may also be capable of bait fishing. The focal individual manipulated and transported pieces of bread in a bait-like fashion and performed several predatory attempts, including one successful strike, on small fish attracted to the floating food. The repeated use of the behaviour and the active manipulation of the bait are consistent with tool use definition.

This observation provides new insights into the behavioural flexibility of gulls and may expand the current list of species reported to employ bait fishing. More broadly, our findings highlight the potential of citizen science approaches for documenting rare behavioural innovations and improving our understanding of animal cognition and behavioural adaptation.

POSTER N° 31

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From exhibition to micro-conservation: new breeding projects at the Aquarium of Natural History Museum, University of Pisa

Freshwater biodiversity is undergoing one of the fastest global declines, making *ex-situ* conservation a critical tool against species loss. Since 2008, the Natural History Museum of the University of Pisa has hosted the largest Italian freshwater aquarium, now functioning both as public exhibition space, and as conservation facility.

Our aquarium started a pilot breeding program focused on five high conservation priority species: *Aphanius fasciatus*, *Palaemon antennarius*, *Allotoca maculata*, *Xenotoca eiseni* and *Xenoophorus captivus*. The latter three Mexican goodeids are listed as Critically Endangered or Endangered. Since April 2025, the Museum has been an active partner in the Allotoca-Mesa Central Project (AMCP), coordinated by the Goodeid Working Group, which aims at securing genetically viable, self-sustaining *ex-situ* populations for future reintroductions.

Our husbandry approach is conservation-oriented, replicating natural habitat to maximize welfare and to promote spontaneous reproduction. Target species are maintained in semi-natural aquaria with gravel/sand bottom, live vegetation, abundant shelters, and seasonal light cycles. Diet includes dry food, live nematodes, and supplementary phytotherapeutic products. Under these conditions, adults reproduce naturally and also fry survival was good: between May 2025 and May 2026, our program produced 15 *A. fasciatus*, 21 *X. eiseni* and over 30 *P. antennarius* raised to adulthood, with additional pregnancies ongoing in the goodeids.

For *P. antennarius*, a species strongly affected by habitat degradation in Italy, we are developing a larval-rearing protocol involving isolation of ovigerous females in a nursery tank, furnished with organic material, until hatching. The first successful rearing attempt was conducted between February and April 2026. Larvae were fed with live micronematodes and powdered food until they grew up to 1.5 cm; juveniles were then transferred back to the exhibition aquaria.

These preliminary results emphasize the potential of targeted *ex-situ* micro-conservation actions within an institutional aquarium. By reducing reliance on native populations in the wild, establishing self-sustaining populations in captivity and refining species-specific breeding protocols, our work contributes to the long-term goals of the AMCP, and to broader efforts to save freshwater biodiversity. Planned outreach initiatives, including a new exhibition tank for goodeids, will also strengthen public engagement, an essential component of successful conservation strategies.

POSTER N° 32

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Network strategies and co-design for inclusive zoology: the experience of ADMaiora, educational services manager at the Municipal Aquarium of Milan

Science communication regarding biodiversity and aquatic conservation must face the challenge of universal accessibility today. The right to information, as established by the ONU Convention on the Rights of Persons with Disabilities (Art. 9), requires that complex zoological content be made accessible to visitors with cognitive or sensory vulnerabilities. The Civic Aquarium of Milan, through its educational department ADMaiora, has implemented a "network" model based on co-design with specialized organizations to break down these barriers. To illustrate the methodologies and tools developed to facilitate the learning of zoology and biodiversity, ensuring autonomy and active participation through a multisensory approach and simplified language. The initiative was structured around three key pillars, each resulting from a specific synergy:

Linguistic Accessibility (Renato Piatti Foundation of Varese): following the European guidelines of Inclusion Europe, an orientation brochure and a social story were created using Easy-to-read (EtR) language. These documents, approved under the ANFFAS quality mark, use short sentences and large fonts to explain the exhibition route and rules of conduct, reducing environmental anxiety and fostering predictability for the visit.

Educational Co-design (Paideia Association): an ADMaiora expert and an operator from the association collaborated to review existing workshops such as "Shark Lab" (biology of elasmobranchs) and "Beach Detective" (exploration of environmental traces). The process involved in-person and online meetings to adapt materials (EtR texts, enlarged fonts) and group management reducing the number of specimens to be observed to prevent cognitive overload and calibrating activities to the needs of neurodivergent children and youths.

Sensory Accessibility (Institute for the Blind in Via Vivaio): to support visually impaired visitors, a kit of tactile bas-relief panels representing marine and freshwater vertebrate and invertebrate animals was developed. During guided tours, these bas-reliefs are integrated with the exploration of natural materials (shells, starfish, egagropili) alongside auditory and olfactory stimuli, promoting a "full-sensory" understanding of nature.

The tools were validated through pilot workshops with groups of approximately 15 people, including families from the Paideia Association, confirming the effectiveness of an approach that integrates AAC (Augmentative and Alternative Communication) signage and sensory maps. The Milan experience demonstrates that inclusive zoology does not compromise scientific rigor; rather, it strengthens the museum’s role as a sanctuary for active citizenship and lifelong learning for all visitors.

POSTER N° 33

Sessione poster

TEMA LIBERO



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Taxa IdentiFAIR: a digital framework for assisted taxonomic identification aligned with FAIR principles

Accurate taxonomic identification represents a critical process across many biological disciplines. However, the knowledge required for species-level determination is frequently confined within limited textual descriptions (books, PDFs, geographically restricted keys), making morphological data difficult to locate and non-interoperable (non-FAIR). Although transitioning toward electronic interactive systems is a path already adopted in the past (e.g., Böttger-Schnack & Schnack, 2015), systematics still requires web architectures capable of integrating global data streams. This contribution introduces Taxa IdentiFAIR, a digital framework powered by React and Supabase, conceived as a universal "skeleton" designed to accommodate, through collaboration with taxonomists, morphological databases of heterogeneous animal taxa.

The core objective of the project is to act as a "FAIRness enabler" for descriptive morphology. The system converts diagnostic features into Findable and Accessible data via an open web interface. This standardized architecture renders the resulting morphological data intrinsically Reusable for computational analyses, both internally with an interactive identification system and externally through other computing systems. Both contextual parameters (such as sex, sampling area, etc.) and standardized anatomical traits can be used for a non-linear identification process by exclusion, wherein users query the system based on the characters visible under the microscope, thereby adopting a “multi-trait” approach to overcome the rigidity of traditional dichotomous keys.

Interoperability is ensured by native integration via APIs with major global infrastructures and databases. The framework does not intend to replace these platforms but relies on them, recognizing their specific, distinct, and irreplaceable scientific roles: it adopts nomenclature from WoRMS, retrieves biogeographical data from GBIF, and connects to specialized records such as the Marine Planktonic Copepods portal by Razouls et al. (2005-2026).

Currently in a prototyping phase focused on Copepoda Calanoida as a demonstrative case study, the roadmap includes the implementation of assisted visual comparison features, the dynamic generation of localized keys, and the integration of AI-based assistance. Taxa IdentiFAIR thus proposes to use digital tools as a bridge between the precision of classical taxonomy and the interoperability requirements of modern Open Science.

POSTER N° 34

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Pine marten (*Martes martes*) in southern Milan: occurrence within a fragmented peri-urban landscape

The pine marten (Martes martes) has traditionally been considered a forest specialist species sensitive to anthropogenic disturbance. Recent studies, however, suggest a greater ecological plasticity, including the ability to exploit fragmented and peri-urban environments. In northern Italy, the species has also been associated with riparian corridors and residual woodland patches embedded within intensively cultivated plains, which may play a key role in shaping its distribution within human-dominated landscapes.

Between 2025 and 2026, a continuous camera-trapping survey was carried out approximately 10 km south of Milan's historic city centre, within the Parco Agricolo Sud Milano, near the municipality of Pieve Emanuele, in a highly fragmented agriculturalurban matrix characterized by narrow canal-side wooded corridors connected to a few isolated woodland patches of only a few hectares.

The survey documented repeated occurrence of the species through a substantial number independent detections including simultaneous records of two individuals, particularly along corridors characterized by mature arboreal vegetation and dense cover, suggesting preferential use of residual ecological connectivity elements within the investigated peri-urban landscape. Among the investigated areas, detections were strongly concentrated along the canal-associated corridor, while only occasional or no records were obtained in the other monitored sites. The monitored areas were also frequented by several other mammals, including red fox, badger, roe deer, wild boar, crested porcupine and red squirrel. Preliminary data analyses suggest a mainly nocturnal activity pattern with crepuscular peaks.

The results confirm the occurrence of the pine marten within the urban and peri-urban landscape of southern Milan and support the hypothesis that narrow linear wooded elements and small remnant woodland patches may contribute to maintaining functional connectivity for forest-associated mammals in highly anthropized lowland environments, highlighting the importance of incorporating peri-urban landscape into future conservation strategies and preserving the last elements of ecological connectivity in highly anthropized lowland environments.

POSTER N° 35

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A source-based annotated checklist of the terrestrial pulmonate gastropods of Sardinia and its satellite islands

Sardinia is an ancient continental island and one of the principal centres of land-snail endemism in the western Mediterranean, surrounded by a constellation of satellite islands that replicate its granitic, volcanic, sedimentary and calcareous substrates under further isolation. Yet this rich fauna remains dispersed across more than a century of taxonomic literature, only partly mobilised into open databases, and very unevenly documented, the satellite islands least of all.

Here we provide the first fully source-referenced annotated checklist of the terrestrial pulmonate Gastropoda of the main island of Sardinia and 7 satellite island units, integrating published literature, museum collection data, the Italian national checklist and verified GBIF (Global Biodiversity Information Facility) occurrence records. The taxa span 37 families; 35 (27.8%) are Sardinian or Tyrrhenian endemics, 21 of them restricted to Sardinia alone. Unlike previous inventories, each taxon-island record is explicitly linked to its documentary source, so that the entire checklist rests on traceable evidence. This source-based treatment exposes a severe and previously unquantified survey gap: 4 of the 7 satellite units are represented by no verified record at all, and each of the remainder by fewer than 10, against the full fauna documented on continental Sardinia. The checklist provides a reproducible baseline for future faunistic, biogeographical and conservation studies, while identifying priority areas for target sampling.

POSTER N° 36

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Effects of cadmium and lead exposure on *Hermetia illucens* larvae

The larvae of the black soldier fly (*Hermetia illucens*, BSFL) are highly efficient bioconversion agents capable of converting a wide range of organic waste streams into valuable biomass. The exploitation of novel rearing substrates that are not yet authorized under European legislation could increase BSFL production while reducing the costs of insect-derived products. Among these substrates, the organic fraction of municipal solid waste (OFMSW) is particularly promising because of its high nutritional value and the large quantities generated worldwide. However, OFMSW may contain chemical and microbiological contaminants, including heavy metals (HMs), which could affect larval growth, insect health, and the safety of BSFL-derived products.

In this study, BSFL were reared on a surrogate OFMSW contaminated with $PbCl_2$ (120 mg/kg dry matter) or $CdCl_2$ (1.6 mg/kg dry matter). Growth performance, survival, and adult emergence were monitored and compared with those of larvae reared on control OFMSW. The activation of the immune system was assessed through cellular (hemocyte counts) and humoral (antimicrobial activity) markers. Moreover, the molecular response to HM exposure was investigated by qPCR analysis of genes involved in metal detoxification and oxidative stress. Finally, lead and cadmium accumulation was quantified by ICP-MS in larvae, larval tissues (midgut epithelium, fat body, hemolymph and Malpighian tubules), frass, and adults.

Our results demonstrated that the exposure to lead and cadmium did not affect the insect life cycle, although cadmium exposure significantly reduced adult emergence. Both metals elicited cellular and humoral responses and induced the upregulation of genes associated with detoxification and oxidative stress pathways. Furthermore, lead and cadmium accumulated in all larval tissues analyzed, as well as in frass and larvae.

Overall, this study provides new insights into physiological and molecular responses of BSFL to heavy metal exposure and highlights potential safety concerns associated with the use of contaminated OFMSW as a rearing substrate for insect production.

POSTER N° 37

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Beyond the labyrinth of Daedalus: multi-species co-habitation and structural complexity in crustacean burrows

The present study provides the first evidence of an extensive burrow system characterizing the soft-bottom habitats of the Mar Piccolo basin (Taranto, Mediterranean Sea), where these structures act as a key organizing element over large seabed areas, representing a key structural element across extensive patches of the Mar Piccolo.

The temporal dynamics and spatial variability of these bioturbation structures were investigated over one year (March 2025 – February 2026) through monthly monitoring of standardized plots delimited by PVC squares and distributed across different areas of the basin. The three-dimensional architecture of the burrow systems was reconstructed using polyester resin polymerized *in situ*, while the burrowing fauna and associated community were characterized by underwater visual censuses and seasonal day- and night-time video recordings.

The decapod crustacean *Alpheus dentipes* was identified as the main burrowing organism, bioturbator and ecosystem engineer, extensively modifying seabed topography. A persistent mutualistic association with the teleost *Gobius niger* was consistently observed, confirming a symbiosis already documented for several gobiid species and burrowing alpheid shrimps. Underwater observations and video analyses further revealed a rich community of associated fauna exploiting the burrow system and its surroundings for shelter and foraging. This community included the stomatopod crustacean *Squilla mantis*, the decapod *Penaeus kerathurus*, several teleost fish (most notably species of Syngnathidae and Gobiidae, as well as *Serranus hepatus*), together with many nudibranchs and other benthic invertebrates. The burrow network exhibited temporal variability, with substantial fluctuations in density and distribution but consistent trends across the surveyed sites. Resin casts revealed a complex three-dimensional architecture composed of interconnected tunnels and highly branched galleries extending over wide areas.

These preliminary results reveal structural complexity in Mar Piccolo soft-bottom habitats and highlight the pivotal ecological role of burrowing crustaceans in transitional ecosystems. By continuously reworking unconsolidated sediments, they contribute to sediment oxygenation and organic matter processing while simultaneously increasing habitat heterogeneity, thereby promoting biodiversity through ecosystem engineering.

POSTER N° 38

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Decoding tardigrade extremotolerance: transcriptomic and functional insights underlying anhydrobiosis and heat stress

Tardigrades are microscopic animals inhabiting nearly all ecosystems on Earth, from marine and freshwater environments to terrestrial mosses and soils, representing an outstanding example of hidden biodiversity. Their ecological success relies on their ability to withstand extreme environmental conditions through highly specialized stress-response mechanisms. Tardigrade-specific intrinsically disordered proteins (TDPs, i.e. cytosolic, secretory, mitochondrial abundant heat soluble proteins CAHS, SAHS, MASH) are a key component associated with desiccation tolerance (anhydrobiosis) and suggested to contribute to thermal stress resistance. However, the molecular basis and functional breadth of these adaptations remain partially understood. This study investigates the molecular mechanisms underlying desiccation and thermal stress tolerance in two tardigrades with different dormancy performances by integrating comparative transcriptomics, functional genomics, and gene expression analyses. In the leaf litter anhydrobiotic species *Paramacrobiotus spatialis*, RNA-seq identified genes associated with anhydrobiosis, evidencing significantly upregulated genes during desiccation: MASH, mitochondrial late embryogenesis abundant protein (LEAM), and ornithine decarboxylase (ODC). A functional RNA interference pipeline has been established to target for silencing each of these genes in order to test whether their knockdown could affect anhydrobiotic survival following desiccation and rehydration. In the freshwater species *D. parthenogeneticus*, desiccation assays showed no detectable survival after a cycle of dehydration/rehydration, whereas thermotolerance experiments demonstrated survival after exposure up to 40 °C. Short-read genome sequencing of this species enabled the identification and annotation of TDP-encoding genes, providing a framework to investigate their role in thermal tolerance. RT-qPCR was used to assess whether TDP expression is modulated under heat stress, clarifying their contribution to a shared stress-response network. By combining comparative transcriptomics with functional and expression-based approaches, this work advances understanding of stress tolerance in tardigrades, highlighting conserved and interconnected mechanisms underlying anhydrobiosis and thermotolerance. These findings may support future applications in biomolecule stabilization and biological preservation.

POSTER N° 39

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Temporal variations in the abundance of *Hippocampus guttulatus* and benthic communities in the Mar Piccolo of Taranto (Ionian Sea, Italy)

Seahorses and other syngnathids are vulnerable fishes due to a combination of biological and ecological traits, including low mobility, high site fidelity, low fecundity, and specialized habitat use. Seahorses are frequently associated with confined coastal environments, where they often reach relatively high densities. In this context, the Mar Piccolo of Taranto represents a particularly important system, as it hosts one of the most abundant known populations of *Hippocampus guttulatus* in the Mediterranean. This study reports data from standardized underwater surveys conducted in 2013, 2021, and 2025 at a long-term monitoring site, aimed at assessing the abundance, sex ratio, size-class structure, and habitat associations of the long-snouted seahorse, *H. guttulatus*. The species exhibited a significant temporal decline in abundance throughout the study period. Sex ratio remained broadly comparable over time, whereas size-class structure differed markedly, with all size classes being well represented only during the first survey. In 2013, the species was primarily associated with vertically structured substrates, such as stone walls and mussel-farm poles, whereas in 2021 and 2025 most individuals were recorded on soft-bottom habitats characterized by scattered biogenic concretions. This change in habitat association was accompanied by shifts in benthic community composition, including the appearance of potential seahorse predators such as the Atlantic blue crab (*Callinectes sapidus*). The decline of *H. guttulatus*, together with the persistence of abundant populations of other syngnathid species (i.e., pipefishes), closely related to seahorses as concerns their main ecological traits suggests that habitat turnover alone may not fully explain the observed patterns. Additional pressures acting selectively on seahorses, including illegal harvesting and trade previously documented in the Mar Piccolo of Taranto, may also have contributed to the observed decline. These findings highlight the importance of considering both habitat dynamics and species-specific vulnerabilities when interpreting long-term population trends and emphasize the need for continued monitoring to assess the spatial extent and drivers of ongoing population changes.

POSTER N° 40

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Preliminary observations on the reproductive effort of *Dendropoma* sp. in Apulia Region (South Italy)

Vermetid bioconstructions are peculiar formations commonly distributed in the intertidal zone along the southern coast of the Western and Eastern Mediterranean Sea. These structures alter local hydrodynamics, stabilizing the substrate and providing essential habitat complexity that supports diverse benthic assemblages. Despite their ecological importance, knowledge of their reproductive biology and cycles are limited to few isolated studies. This preliminary study reports the reproductive effort of the vermetid *Dendropoma* sp. along the Apulian coasts during the period April–September 2025. Selected sampling sites were Monticelli in province of Brindisi for the Adriatic Sea and Taranto for the Ionian sector. At each site, small rock fragments containing vermetid concretions were collected and placed in containers filled with seawater. In the laboratory ten specimens per site were gently removed from the rocky substrates and observed. These specimens were firstly photographed under stereomicroscopy to assess their morphology and the presence of egg capsules. Using optical microscopy, gametogenic status was detected by assessing the presence of sperm cysts or oocytes/embryos.

Data about the studied period reveal spatial heterogeneity in reproductive effort indicators. In the Adriatic Sea (Monticelli, BR), female predominance emerges from April to June. The presence of formed protoconchs is observable from April to June. Males appear mainly in May, becoming occasional between June and July. This trend indicates a reproductive peak between late spring and early summer, followed by residual gametogenic activity until late summer.

In the Ionian Sea (Taranto), early spring shows a mixed sex distribution, with females observed from April to August and protoconchs from May to July, with a reproductive peak in May. Males are sporadic and observed from April to July. Most are indeterminate, suggesting a prolonged or staggered spawning/recruitment dynamic in the Ionian sector. In both basins, combined evidence supports a pronounced spatiotemporal pattern, with increased reproductive effort in late spring, continued activity until midsummer and reduced or undetected activity in September, mirroring the reproductive pattern described for other vermetid species in the Mediterranean. Further observation covering the remaining months of the year are required to fully understand the complete reproductive cycle of this species.

POSTER N° 41

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Seahorses as living substrates? Insights into epibiosis from the Mar Piccolo of Taranto (Ionian Sea, Italy)

Seahorses are characterized by high site fidelity and a predominantly sedentary lifestyle, using their prehensile tail to grasp a variety of natural holdfasts. These behavioural traits bring them into continuous contact with benthic invertebrates and algae. A few isolated observations have reported epibiotic organisms on seahorses and pipefishes, indicating that colonization of syngnathids by sessile organisms can occur. However, the prevalence of these associations in natural populations and the diversity of the organisms involved remain virtually unknown. Here, we provide preliminary quantitative information on the occurrence of epibiosis in two Mediterranean seahorse species from the Mar Piccolo of Taranto.

Between May and July 2025, 109 seahorses, including 78 *Hippocampus guttulatus* and 31 *H. hippocampus,* were photographed *in situ*, and the images were subsequently examined for the presence of epibionts. Evidence of epibiosis was observed in 35 *H. guttulatus* (44.9%) and 14 *H. hippocampus* (45.2%), corresponding to 49 individuals in total (45.0%). A further 20 individuals carried only detrital material, while 40 showed neither epibionts nor detritus. Epibionts belonged to different taxonomic groups, including brown algae, bryozoans, encrusting algae, hydrozoans, barnacles and sea anemones. Brown algae were the most frequent epibiont taxa, usually covering extensive portions of the seahorse body surface, whereas hydrozoans and sea anemones were recorded only in a few individuals.

Our observations suggest that epibiosis may be more common in Mediterranean seahorses than currently documented. The diversity of epibiont taxa recorded suggests that seahorses may act as substrates for a variety of sessile organisms. While these associations may simply reflect opportunistic colonization, they could also promote passive dispersal of sessile organisms through host movements. Conversely, epibionts may contribute to the cryptic appearance of the host, although this hypothesis remains entirely unexplored. Our observations provide one of the first quantitative descriptions of epibiosis in Mediterranean seahorses and establish a baseline for future studies combining broader spatial and temporal sampling with integrative morphological and molecular identification of epibiotic assemblages to clarify the ecological significance of these interactions.

POSTER N° 42

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New tools for zoological research: the mesocosms of the alpstream center

Alpine rivers represent ecosystems of exceptional biodiversity, biogeographic, and hydrological significance, characterized by numerous endemic species that are highly adapted to harsh environmental conditions and are often threatened by climate change and human activities. Extreme environmental and seasonal variability, logistical constraints, and related factors make conducting research in these environments particularly challenging, often resulting in studies that are primarily descriptive. In this context, mesocosms are valuable tools for zoological studies, allowing manipulative experiments under semi-natural conditions while maintaining control over key environmental variables. We present here the ALPSTREAM bio-hydraulic laboratory, a facility designed to reproduce alpine stream conditions and support biodiversity and conservation research. ALPSTREAM is located in Ostana (NW Italy), within the Monviso Biosphere Reserve and Monviso Natural Park, and is supplied directly by the Po River headwaters through a gravity-fed system. The facility successfully reproduces realistic stream conditions while enabling controlled manipulation of hydraulic and biological variables. The facility comprises six experimental flumes (25 m long) with adjustable hydraulic controls and natural substrates reproducing alpine stream habitats. Some preliminary studies have already been carried out, investigating, for example, the trophic behaviour of some Heptageniidae (Ephemeroptera) and its impact on periphyton dynamics, the potential for ex situ breeding of the threatened European bullhead (*Cottus gobio* L., 1758), the application of eDNA techniques, and the effects of flow variations on benthic invertebrates, benthic fish, and urodele larvae. Despite limitations inherent to mesocosm approaches, including reduced spatial complexity and hydrological connectivity, ALPSTREAM represents a significant addition to the extremely limited network of alpine stream experimental facilities. It provides a robust platform for studying biodiversity responses to environmental change, testing restoration measures, and conducting multi-stressor experiments under controlled yet realistic conditions. By bridging laboratory and field approaches, ALPSTREAM can support both fundamental research and evidence-based management of mountain river ecosystems. Our center is part of the Mesocosm.org network, a worldwide network of aquatic mesocosm facilities.

POSTER N° 43

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Reproductive timing of *Paracentrotus lividus* in Southern Apulia: preliminary evidence from Gonadosomatic-Index monitoring

The sea urchin *Paracentrotus lividus* is a key species in Mediterranean benthic ecosystems and a commercially valuable resource. Intensive exploitation has caused the decline of natural stocks in several areas of the Mediterranean Sea. In response to this issue, in 2023 the Apulia Region introduced a three-year ban on the collection of local specimens (Regional Law No. 6, 18/04/23), highlighting the urgent need for updated scientific data on the reproductive biology of the species to validate or revise the national fishing closure period (May-June), established by Ministerial Decree 12/01/1995, which may no longer align with current biological cycles in the context of climate change.

This study presents preliminary results from research conducted in the area of Santa Caterina (Lecce, Italy), aimed at monitoring the seasonal variation of the Gonadosomatic Index (GSI) as a proxy for the reproductive status of the population.

Each month, 60 individuals in total, 20 per site, with a test diameter without spines (TD > 35 mm), were collected and analysed for morphometric parameters, sex, and GSI. The relationship between GSI and the main environmental drivers, known to influence the reproductive dynamics of the species, was examined.

Preliminary data from the first five months of sampling (February-June 2026) showed significant temporal variation in GSI (Kruskal-Wallis: $\chi^2 = 65.392$, df = 4, $p < 0.001$). The highest values were recorded in February (4.40 ± 0.24%) and March (4.47 ± 0.24%), followed by a progressive decrease until June, when the lowest mean value was observed (2.54 ± 0.19%).

Mean monthly GSI was negatively correlated with temperature, photoperiod (Spearman: $\rho = -0.90$, $p = 0.0374$ for both variables), and chlorophyll-a (Spearman: $\rho = -1$, $p < 0.001$), whereas salinity and dissolved oxygen showed no significant effects.

No significant differences in GSI were detected between sexes (Wilcoxon-Mann-Whitney: $W = 9011$, $p = 0.2928$), while larger individuals showed greater reproductive investment (Kruskal-Wallis: $\chi^2 = 22.203$, df = 2, $p < 0.001$), confirming their key role in the reproductive potential of the population.

Overall, these preliminary results support the hypothesis of earlier gonadal maturation and a possible shift in the reproductive period compared with the current national protection window, providing a scientific basis for more effective and sustainable management of this resource in the Apulia region.

POSTER N° 44

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Generation of improved black soldier fly larvae for polyethylene terephthalate (PET) degradation

The larvae of the black soldier fly (*Hermetia illucens*, BSFL) are increasingly recognized as an efficient platform for the valorization of organic waste and agro-industrial by-products thanks to their saprophagous feeding behavior and remarkable bioconversion efficiency. However, these substrates are often contaminated by recalcitrant pollutants, including microplastics (MPs), which may compromise the sustainability and safety of the waste valorization process. Among MPs, polyethylene terephthalate (PET) is one of the most used and recalcitrant plastic polymers.

In this study, we generated a genetically engineered line of *H. illucens* expressing the F243T/C292A-ΔLCC (TA-ΔLCC) variant of the thermostable PET-degrading enzyme leaf-branch compost cutinase (LCC), potentially enabling BSFL to degrade PET. To this end, BSF embryos were microinjected and the TA-ΔLCC expression cassette was integrated into the genome using a piggyBac-mediated approach. Transgenic larvae were subsequently characterized by analyzing transgene expression, protein localization in the larval midgut, and PET-degrading activity using both *in vitro* and *in vivo* assays.

The engineered larvae showed high expression levels of TA-ΔLCC in the anterior, middle, and posterior midgut, with protein accumulation detected in the posterior midgut. *Ex vivo* and *in vivo* assays confirmed PET-degrading activity in the midgut. Importantly, no alterations in midgut morphology and physiology were observed, indicating that transgene expression did not adversely affect gut functionality.

Overall, our results demonstrate the successful generation of transgenic BSFL capable of expressing a functional improved variant of the cutinase LCC, the reference enzyme for industrial applications, in the midgut. This work expands the potential of *H. illucens* as an innovative biotechnological platform for the treatment of organic waste streams contaminated with persistent synthetic polymers and represents a significant step toward the development of insect-based solutions for plastic bioremediation.

POSTER N° 45

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New records of the demosponge *Calyx nicaeensis* (Risso, 1827) along Apulian coasts (southern Adriatic Sea)

In recent decades, some iconic species of the sessile macrozoobenthos of the Mediterranean Sea have undergone a drastic reduction in abundance values. The causes are mainly to be found in the increase in sea water temperature and the consequent appearance of opportunistic pathogens. Furthermore, the synergy with other anthropogenic impacts on the coastal marine environment, such as increased sedimentation rates, has caused a change in conditions once favorable to the growth and reproduction of a large number of filter-feeding organisms, such as Porifera. Among these, the demosponge *Calyx nicaeensis*, endemic to the Mediterranean Sea and classified by the IUCN as critically endangered. It is a massive sponge with a characteristic cup-like shape.

In Apulia, over the last 20 years records of this species have been rather sporadic: as reported by Cerrano et al. (2014), Longo et al. (2018) and Trainito et al. (2020), the sponge is documented mainly along the Ionian coasts of Salento, with some isolated specimens in Porto Cesareo, on coralligenous bottoms at about 30 m depth; 1 specimen in Nardò, in a semi-submerged cave at 3 m depth; 2 specimens in Otranto, on photophilous bottoms at depths between 11 and 18 m; some specimens in Santa Caterina di Nardò, on photophilous bottoms at 20 m and some specimens in Santa Maria al Bagno and Torre Inserraglio, on photophilous bottoms at depths between 25 and 30 m.

Over the past five years, ROV surveys conducted as part of the Marine Strategy and aimed at monitoring the Apulian coralligenous habitat have added new records of *C. nicaeensis* to the existing data: one specimen was found in the waters off the city of Bari, at a depth of 28 meters; four specimens off Mola di Bari, at a depth of approximately 18 meters; four specimens off Monopoli (Bari), at a depth of approximately 20 meters, and three specimens in the waters off Alimini (Lecce), at a depth of 35 meters. These new records extend the species' range to the Italian coasts of the Southern Adriatic, confirming its extremely fragmented distribution and low density values, and highlighting the urgent need for adequate conservation measures.

POSTER N° 46

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Millennial persistence of freshwater gastrotrich DNA in ancient pond sediments revealed by metabarcoding

Freshwater gastrotrichs are microscopic meiofaunal metazoans whose diversity is still poorly documented and whose representation in sedimentary ancient DNA (sedaDNA) studies remains largely unexplored. Here, we investigated whether DNA metabarcoding can recover gastrotrich signals from ancient freshwater sediments across millennial timescales, combining zoology, paleoecology, palynology, molecular biology, and bioinformatics. A sediment core was collected from a dried pond within the Castelporziano Presidential Estate (Rome, Italy) and analysed at three dated horizons spanning the last ~5700 years (489, 1403, and 5690 calibrated years BP). Palynological evidence linked all three layers to freshwater wetland conditions through the presence of pollen from plant taxa characteristic of these habitats. DNA metabarcoding targeted two mini-barcode markers of the 18S rRNA and mtCOI genes. Taxonomic assignment was enhanced using a customized reference database combining publicly available sequences with additional freshwater gastrotrich sequences generated in our laboratory. All gastrotrich ASVs recovered from the three layers belonged to freshwater taxa, fully consistent with the paleoenvironmental reconstruction based on palynological data. In total, 34 ASVs were assigned to Gastrotricha, of which 21 were identified to genus or species level using the customized database. The oldest layer displayed the highest genetic diversity. Notably, gastrotrich DNA was recovered from sediments dated to 5690 years BP, demonstrating the remarkable preservation potential of sedaDNA from freshwater meiofauna over millennial timescales. This finding raises the possibility that resistant life stages, such as resting eggs, may contribute to long-term DNA persistence, although further studies are needed to test this hypothesis. Overall, our results open new perspectives for reconstructing the long-term dynamics of overlooked freshwater meiofaunal communities and highlight the value of integrative zoology in extending biodiversity research beyond present-day ecosystems.

POSTER N° 47

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Mesocosms as a useful tool to investigate freshwater fauna responses to droughts

Droughts and hydrological intermittence are expanding globally, with profound effects on freshwater biodiversity and ecosystem functioning. Understanding the responses of riverine fauna to droughts is essential; however, the continuous environmental changes occurring across the hydrological phases (flowing, drying/dry, and rewetting) make the interpretation of ecological responses complex, limiting our understanding of ecohydrological processes.

In this context, mesocosms represent a valuable experimental tool because they reproduce natural ecosystems while balancing control, replication, and physical realism. They also allow manipulation of environmental and hydrological variables such as drying frequency and intensity. Here we conducted a comprehensive literature review, showing that a wide array of mesocosms has been used over the past decades to investigate numerous biotic responses to drought, spanning from taxa survival and autecological studies, to community-level responses, to single or multiple stressors in intermittent rivers. Mesocosms have been particularly effective in clarifying the effects on macroinvertebrate communities, highlighting processes such as environmental filtering, biodiversity loss, and shifts in functional composition along gradients of drought intensity and duration. Mesocosms have also enabled the identification of critical thresholds of habitat loss and the tracking of community recovery trajectories during rewetting phases. Particularly relevant are studies focusing on hyporheic fauna, which have demonstrated the role of vertical movements into subsurface layers as a resistance mechanism for many benthic taxa. Similarly, controlled experiments have provided key insights into the behavioural and physiological strategies of fish and crayfish, showing that responses to drought are highly species-specific and strongly influence population resilience. Overall, mesocosms emerge as a key tool for understanding the mechanisms governing resistance and resilience of freshwater fauna in intermittent rivers. The possibility to integrate environmental realism, experimental replication, and controlled manipulation of disturbance factors makes mesocosms particularly promising for bridging knowledge gaps about biotic responses to droughts and developing conservation strategies in the context of increasing river intermittency.

POSTER N° 48

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UV, Bioplastic, and Sandhoppers

For the sandhopper *Talitrus saltator* (Montagu, 1808), starch-blended bioplastic became less palatable depending on the amount of starch in its composition: if the amount of starch is reduced, the bioplastic is less consumed. Moreover, the same bioplastic exposed to UV irradiation modifies the starch content and is consumed less than the same bioplastic not irradiated. The consumption of bioplastic by the sandhoppers changes its size from macroplastic to micro/submicroplastic. Moreover, the intestinal transit of the starch-blended bioplastic also modifies its composition. In fact, not only is the starch component digested, but the observed alterations are typical of oxidative or enzymatic degradation processes in the polymers and suggest the possibility of PET degradation.

POSTER N° 49

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Preliminary reports on immune priming in the colonial ascidian *Botryllus schlosseri*

Immune priming is the capability of the innate immune system to change the immune response following a previous experience with nonself molecules. It acts as a form of immune memory in invertebrates, differing from the long-term, lymphocyte-based, immune memory of jawed vertebrates. Immune priming can occur in the form of a “recall” response or potentiation, so that the second response is faster and more powerful than the first one, as a shift from one type of response to another, more efficient response, as a long-lasting upregulation of the defence response, or even as a state of tolerance with reduced immune activity.

First evidence in the scientific literature points towards the possible presence of immune priming in colonial ascidians. The few available data refer to the possibility, in *Botryllus primigenus* and *Botryllus schlosseri*, to alter colony fusibility when colonies were previously fused, for a few days, to a compatible colony sharing only one allele at the Fu/HC locus. The phenomenon is due to the persistence, in a colony, of a cell population from the previously fused colony.

With the present work, in the attempt to demonstrate the presence of immune memory in colonial ascidians, we primed colonies of *B. schlosseri* by microinjecting bacteria or LPS from *E. coli* in the colonial vasculature and compared the obtained response, in terms of transcription of immune-related genes, to that obtained following a second challenge. The responses in various phases of the colonial blastogenetic cycle were also considered. Results indicate the capability of *B. schlosseri* to enhance the transcription of immune-related genes after the second challenge and pave the way for further analyses on the molecular mechanism(s) underlying this phenomenon.

POSTER N° 50

Sessione poster

Workshop - Modelli invertebrati: una reale opportunità nell'applicazione del principio delle 3R

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The medicinal leech *Hirudo verbana* as a valid ecotoxicological model for assessing the biological effects of emerging aquatic contaminants

The increasing release of emerging contaminants into aquatic ecosystems poses a significant threat to environmental health, highlighting the need for reliable ecotoxicological models capable of assessing both organismal and ecosystem-level responses. Among these, microplastics (MPs) and nanoplastics (NPs), synthetic fragrances such as Galaxolide, and per- and polyfluoroalkyl substances (PFAS) are of particular concern due to their widespread occurrence, environmental persistence, and potential for bioaccumulation. Although their dispersion and ecological impact have been already documented, the molecular mechanisms underlying their toxic effects remain often poorly understood. With this objective, the medicinal leech *Hirudo verbana* represents a promising complementary model organism for ecotoxicological investigations. Owing to its sensitivity to environmental stressors, well-characterized immune system, and ease of laboratory maintenance, this organism provides a valuable platform for evaluating the biological effects of aquatic pollutants. Moreover, its close interaction with freshwater sediments and water makes it particularly suitable for monitoring contaminant exposure under environmentally relevant conditions. In this context, using an integrated approach combining morphological, immunological, and molecular analyses, our recent studies demonstrated that exposure to emerging contaminants induces in leeches alterations in immune cell activation, oxidative stress, inflammatory responses, and metabolic processes, demonstrating that this *in vivo* model can provide mechanistic useful insights into their modes of action. Overall, the medicinal leech emerges as a sensitive and versatile sentinel organism for freshwater ecotoxicology, contributing to the development of integrated approaches for environmental risk assessment and supporting the implementation of strategies aimed at protecting aquatic ecosystems from the increasing burden of emerging contaminants.

POSTER N° 51

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The medicinal leech *Hirudo verbana*: an in vivo model for biomaterial evaluation in wound healing and regeneration

The medicinal leech *Hirudo verbana* serves as an invaluable in vivo model for investigating the complex interplay between tissue repair mechanisms and innate immune responses, thanks to its remarkable regenerative capacity and evolutionary conserved defense pathways. In this study, we utilized *H. verbana* to assess the efficacy and biocompatibility of novel wound-care biomaterials within a living organism.

We challenged the regenerative system of *H. verbana* by implanting two distinct 3D patches, a porous collagen-based scaffold and a 3D-printed gelatin-based patch, into injured tissues. By monitoring the hosts at 72 hours and 1 week post-implantation, we leveraged the leech's biological responses to evaluate patch integration, cell colonization, and the modulation of local innate immune components, which are pivotal for both wound closure and antimicrobial defense. Histological, immunohistochemical, and ultrastructural analyses demonstrated that *H. verbana* provides a robust and sensitive platform for exploring the evolutionarily conserved links between tissue regeneration and immune function. Ultimately, this approach validates the use of *H. verbana* as a highly effective model to rigorously assess the regenerative potential of advanced biomaterials in a comprehensive, whole-organism context.

POSTER N° 52

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An applied example of the 3Rs in cephalopod research: age estimation in *Sepia officinalis* paralarvae

This work offers an applied example of the 3Rs principles (Replacement, Reduction, Refinement) in research on a marine invertebrate model, *Sepia officinalis* (Linnaeus, 1758). The project relies exclusively on paralarvae obtained from egg clutches accidentally found on commercial fishing gear, thereby avoiding the collection of breeding adults and any induced reproduction in captivity. This approach also enables the use of biological material otherwise destined for certain mortality, thereby reducing the overall impact of research on natural populations.

The study focuses on age determination during early developmental stages through the analysis of hard structures (beak, statoliths, and cuttlebone). Since age estimation in adult individuals has already been investigated, acquiring accurate data at early stages using individuals reared under controlled conditions with known chronological age allows the current knowledge gap to be addressed and age estimation methods to be validated across the entire life cycle of the species. All procedures will be conducted in the accredited marine organism facility of the Department of Biology, University of Naples Federico II, in compliance with Directive 2010/63/EU and Italian Legislative Decree 26/2014.

Eggs will be incubated in rearing tanks under controlled temperature and salinity conditions (17.6 ± 2.6 °C; 36.8 ± 0.9 ppm) until hatching. Paralarvae will be monitored daily and maintained, following established protocols, at 20.4 ± 1.7 °C and 37.2 ± 1.17 ppm, and fed ad libitum with live *Artemia salina*. Sampling for hard structure extraction will be carried out at fixed intervals (every 3–5 days), including individuals that die naturally during the rearing period.

Preliminary observations indicate that hatching occurs under controlled conditions, and analyses of extracted hard structures, specifically beaks and statoliths, reveal distinct concentric growth increments corresponding to age, up to 22 days post-hatching. In this context, Replacement is partially addressed through the use of cephalopods at an early larval stage; Reduction through opportunistic egg recovery and an optimized experimental design; Refinement through non-invasive protocols and the final release into the natural environment of the surviving paralarvae. The expected outcomes will advance knowledge of cephalopod biology and support sustainable marine resource management, representing a transferable example of 3Rs implementation in marine invertebrate research.

POSTER N° 53

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Hirudo verbana as a promising invertebrate model for the preclinical evaluation of cell-free regenerative therapies: advancing the 3Rs principle

The identification of ethically sustainable and biologically relevant animal models remains a critical challenge for the validation of emerging cell-free regenerative strategies. In this context, the medicinal leech *Hirudo verbana* emerges as an ideal invertebrate model for studying complex wound healing processes, owing to the evolutionary conservation of key mechanisms, including immune cell recruitment, extracellular matrix (ECM) remodelling, and tissue repair.

This study aims to validate *H. verbana* as a robust *in vivo* platform for assessing the efficacy of human Dental Pulp Stem Cell-derived secretome (hDPSC-S), a promising therapeutic tool for treating chronic wounds and age-related degenerative disorders. Adult leeches were subjected to standardized surgical injuries and treated with either hDPSC-S or a control medium (CTRL). The progression of tissue regeneration was systematically monitored at 48h, 72h, and 1 week post-treatment through a multidisciplinary approach combining morphological, immunohistochemical, and molecular analyses.

Our results demonstrate that the *H. verbana* model responds in a highly sensitive and reproducible manner to exogenous regenerative stimuli, showing significantly accelerated wound healing in the treated group compared to controls. This study confirms that *H. verbana* provides a reliable experimental framework for the preclinical evaluation of regenerative therapies. By integrating this model into biomedical research, we can effectively implement the 3Rs principles, reducing reliance on higher vertebrate models without compromising the quality or depth of biological data.

POSTER N° 54

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Cognitive stimulation as a strategy to improve the welfare of *Octopus vulgaris* in a controlled environment

Improving the welfare of cephalopods maintained in controlled environments represents a significant challenge due to their complex behavioural repertoire, advanced sensory abilities, and need for environmental interaction. Providing opportunities for active exploration and cognitive engagement is essential to support species-specific behaviours and promote positive welfare conditions. In this study, we developed a cognitive stimulation protocol designed to enhance the welfare of *Octopus vulgaris* by promoting exploration, problem-solving, and active interaction with the environment. A novel interactive device was developed to provide octopuses with the opportunity to engage with their surroundings through a controlled problem-solving task. The device consisted of three completely transparent boxes equipped with removable lids, allowing animals to explore and manipulate the structure using their arms. The design promoted the integration of different sensory modalities, challenging the animals to find solutions for their life. The protocol included a habituation, during which animals learned to associate the device with a positive reward, followed by a discrimination between real and artificial prey. Behavioural responses were remotely recorded to minimize human disturbance and analysed through ethological assessments focused on exploration, interaction with the device and problem-solving behaviour. Throughout the discrimination period, *O. vulgaris* showed a progressive reduction in solution time, increased confidence toward the device and more efficient exploration strategies. Animals reduced avoidance behaviours and maintained interaction with the device after obtaining the reward, suggesting increased engagement with the environment. During the cognitive challenge, octopuses modified their behaviour according to feedback, reducing ineffective responses and demonstrating behavioural flexibility. These results suggest that cognitive stimulation through interactive problem-solving tasks can represent a valuable approach to improve the welfare of *O. vulgaris* in controlled environments. Providing opportunities for exploration, manipulation and decision-making may support the expression of natural behaviours and contribute to the development of more stimulating and biologically appropriate conditions.

POSTER N° 55

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The medicinal leech *Hirudo verbana* as an emerging animal model for the production of invertebrate cell lines with innovative biotechnological potential

Medicinal leeches are emerging as valuable experimental models for comparative biology, regenerative medicine, and biotechnology. While mammalian models currently dominate cell-based research despite representing only 0.4% of extant metazoans, aquatic invertebrates encompass the greatest biodiversity on Earth. Nevertheless, stable invertebrate cell lines are currently available only for molluscs and crustaceans. This gap significantly limits the exploitation of these organisms as alternative experimental models.

Over the last two decades, our laboratory has extensively characterized the cellular repertoire of medicinal leeches, identifying hemopoietic precursor cells, monocytes/macrophages, granulocytes, fibroblasts, telocytes, myofibroblasts, and myoendothelial cells. These cell populations display remarkable morphofunctional, and molecular similarities with their vertebrate counterparts, confirming the high evolutionary conservation of fundamental biological processes. Furthermore, using an innovative *in vivo/vitro* approach based on the injection into the leech body wall of Matrigel (MG) biopolymer supplemented with selected cytokines and growth factors, we successfully recruited, isolated and expanded different cell populations.

Starting from these promising results, we propose the medicinal leech *Hirudo verbana*, whose experimental use is not subjected to significant legislative restrictions, as a new emerging model for the production of invertebrate cell lines endowed with innovative biotechnological potential. To this aim, using the MG biopolymer combined with the cytokines PDGF (Platelet-Derived Growth Factor) and EGF (Epidermal growth factor) we have isolated different leech cell populations and cultured them *in vitro* in a culture medium containing basic Fibroblast Growth Factor (bFGF), Transforming Growth Factor- β (TGF-β) and the ribonuclease enzyme *Hv*RNASET2. The responses have been evaluated through morphological and immunocytochemical analyses. In parallel, we generated, for the first time, a pEGFP-N1 expression vector carrying the *H. verbana* Actin 1 minimal promoter to perform transfection experiments aimed at establishing immortalized leech cell lines.

Beyond their intrinsic value for comparative cell biology, these novel cell lines represent a promising platform for the production of bioactive molecules, ecotoxicological assays, and the development of *alternative in vitro* systems capable of complementing or reducing the use of vertebrate models.

POSTER N° 56

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First database of invertebrate species for scientific research: the contribution of UZI to partial replacement of vertebrate models and implementation of the 3Rs principles

The use of animals in scientific research remains essential for advancing knowledge and generating results that benefit human, animal, and environmental health.

However, growing ethical concerns and increasingly stringent regulations, including Directive 2010/63/EU and its national implementations, have stimulated the development of alternative experimental models, particularly as substitutes for vertebrate species.

In response to this need, the scientific community has intensified efforts to identify emerging invertebrate species that may serve as reliable experimental models, contributing to the implementation of the 3Rs principles (Replacement, Reduction, and Refinement), while maintaining scientific validity and reproducibility.

Within this framework, the Italian Zoological Union (UZI) has promoted the development of the first database dedicated to invertebrate species currently used or potentially suitable for scientific research. Freely accessible through the UZI website at the link https://www.uzionlus.it/news/3002-bioetica-e-benessere-animale.html, the database is continuously updated by experts in the field and provides comprehensive information on species biology, laboratory maintenance requirements, and their potential applicability as experimental models. This initiative also highlights the need for greater protocol standardization and formal regulatory recognition concerning the use of invertebrates in research.

In particular, the development of specific guidelines addressing husbandry practices, welfare requirements, and experimental procedures for the most used laboratory invertebrates would represent an important step toward harmonizing research practices.

Pending the establishment of a dedicated European regulatory framework that explicitly includes invertebrates within harm–benefit assessments and the application of the 3Rs principles, UZI contributes by collecting, organizing, and disseminating validated methodologies and updated knowledge. This effort aims to improve animal management and welfare practices, enhance the reliability and comparability of experimental outcomes, and support the broader implementation of partial replacement strategies in scientific research.

POSTER N° 57

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