Sessione
Il pianeta condiviso

Comunicazioni

Alone in the park Facing the biodiversity crisis

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Data speaks loudly: we are facing a monumental biodiversity crisis, and we are the only responsible. We must be clear: this is not completely new. During the existence of our species there have been several times in which we drove to the extinction even large faunas (such as the case of South America's endemic mammals during Cenozoic). However, nowadays our impact is global and there are not completely safe areas, and the actual impact rate is so fast that, for the first time, we can measure population decline in many species and loss of biodiversity along the lifetime of a single human being.
Scientists are yelling since years about habitat loss and degradation, global warming, pollution, resource overexploitation, invasive species, dramatic reduction of ecosystemic services.
Simply, it is not enough. But what can we do, as scientists and as citizens?
Quoting Albert Einstein, *we cannot solve our problems with the same thinking we used when we created them.* We must begin being truly aware that humans are the problem, but we are also the only possible solution. We cannot save ourselves alone because we are stronger together.

Effects of noise pollution on the colonial ascidian *Clavelina lepadiformis*

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Recently, anthropogenic activities had significantly increased noise levels in marine environments, raising the attention about their effects on marine life (Camerlenghi, 2021). Now underwater noise is recognized as an emerging pollutant but is impact on animal life remains poorly understood, particularly in invertebrates. Most studies have focused on vertebrates, leaving a knowledge gap about noise effects on sessile invertebrates.

In our study, we used the colonial ascidian *Clavelina lepadiformis* as a model organism for investigating the consequences of noise exposure under controlled laboratory conditions. Ascidians are filter-feeding tunicates, the sister group of vertebrates, and represent a key component of sea benthic communities.

As adults, *C. lepadiformis*, like other colonial tunicates, can reproduce both asexually, by budding from the stolon or from the body wall of the zooid which helps expanding and maintaining the colony, and sexually, from internal fertilization of eggs that develop into lecithotrophic larvae (Berrill, 1950)

To explore the effects of acoustic pollution, we set out a series of laboratory experiments focusing on different life stages of *C. lepadiformis.* We exposed the animals to a nature-like noise recorded in the environment in water filled tanks using an underwater speaker.

Firstly, we conducted an analysis of oxidative stress in adult individuals collected from both noise-exposed and control tanks after 3 and 6 days. In addition, juvenile individuals were monitored at different time exposure (24h -10 days of treatment) to evaluate cardiac activity as stress response. Moreover, we analyzed the filtration capacity of adult zooids by comparing noise-exposed individuals with untreated controls (1h-24h of treatment).

These multidisciplinary approaches, combining behavioral, physiological, and functional parameters, underline the sensitivity of sessile invertebrates like *C. lepadiformis* to acoustic disturbance. Further investigations will be essential to better understand the ecological implications of noise pollution on benthic communities and to evaluate the long-term consequences on their population dynamics and ecosystem roles.

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Tracking cats through time: ancient DNA and isotopes reveal feline lives in Viking and medieval Germany

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Understanding how domestic animals adapted to and moved within human landscapes is essential for reconstructing past socio-ecological systems. Given their long history alongside humans as pest-control agents and companions, domestic cats (*Felis catus*) offer a unique window into daily life, food economies, and human connectivity across time and space.

This study investigates the genomic background and dietary habits of over 90 cats recovered from two urban contexts in northern Germany: the Viking site of Haithabu (8th–11th centuries AD) and the medieval town of Schleswig (11th–13th centuries AD). Drawing on one of the largest archaeogenetic datasets of domestic cats to date, this work offers valuable insights into feline population dynamics over several centuries. Located in a strategic position with natural protection and navigational access, both sites played a central role in regional and long-distance trade routes that linked northern Europe to wider economic networks during the Middle Ages. Their well-preserved stratigraphy and rich faunal assemblages make them ideal case studies for exploring long-term human-animal interactions.

Despite chronological and socio-economic differences between the two sites, mitochondrial DNA data revealed a shared genetic ancestry across all individuals - assigned to clade IV (*Felis lybica lybica*), specifically haplogroups A, C, and D - suggesting a long-established and relatively stable maternal gene pool in the region likely rooted in dispersal processes starting from the Roman period. The high mtDNA genetic variation was likely shaped by the influx of cats from different regions via trade networks centered on these key European commercial hubs. Nuclear data support minimal admixture with European wildcats (*Felis silvestris*), in line with previous findings and reinforcing the dominance of domesticated lineages.

Stable carbon (^{13}C µ = -19.1 ± 0.7‰) and nitrogen (^{15}N µ = 10.5 ± 1.1‰) isotope data indicate varied trophic behaviors, with most cats showing mixed diets including marine-derived protein reflecting human subsistence strategies and the cats' opportunistic access to food sources.

By combining genetic and isotopic data, this research highlights the ecological flexibility of domestic cats and their integration within evolving urban networks. These animals serve as sensitive bioproxies for tracing cultural trajectories during a period of intense transformation in northern Europe.

Anthropogenic impacts on insect-microbe relationships: the case of herbicides

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The long-standing coexistence between humans and other species has been increasingly shaped by intensive land use and chemical management practices, such as the widespread application of herbicides. While designed to act selectively on plant physiology, herbicides often permeate ecosystems, where they can disrupt ecological and evolutionary dynamics among non-target organisms. Insects, in particular, are embedded in symbiotic networks with microbial partners that are essential to their development, immune function, and survival. These relationships, ranging from mutualism to pathogenicity, form a fundamental axis of insect ecology.

This study investigates how herbicide contamination interferes with insect-microbe interactions, focusing on host-pathogen dynamics in two model beetle species. In *Tenebrio molitor* adults, chronic exposure to a pendimethalin-based herbicide (residual doses: 0.05–13 ppm) altered immune responses to the entomopathogenic fungus *Beauveria bassiana*, evidenced by upregulation of antimicrobial peptides, despite no effect on host survival. Notably, herbicide exposure also inhibited fungal growth. In *Tribolium castaneum* larvae, similar levels of exposure increased susceptibility to the natural pathogen *Bacillus thuringiensis*, resulting in delayed development and reduced pupation success. These findings highlight how human-induced environmental change (via agrochemical use) can destabilize insect-microbe relationships and reshape host-pathogen dynamics. By disrupting symbiotic equilibriums, such contaminants may drive unforeseen ecological and evolutionary consequences. This work underscores the need to integrate microbial symbioses into ecological risk assessments.

Tuna's tale through museomics: millennia of evolution and adaptation between Mediterranean human coastal populations and Atlantic bluefin tuna

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For millennia, Mediterranean coastal communities have relied on fishing as a primary source of sustenance, with evidence of this practice dating back 40,000 years. Understanding the historical extent of human dependence on coastal resources is therefore essential for assessing long-term socio-economic development, human health, and the anthropogenic impact on the environment. Among the apex predators of the Mediterranean Sea, the Atlantic bluefin tuna (*Thunnus thynnus*) holds a prominent position, serving as both a keystone species and a vital resource for traditional sustenance. With few natural predators aside from humans, its exploitation was historically related to subsistence and small-scale commercial fisheries, primarily conducted by coastal and island communities using traditional methods such as *tonnare*. This study aims to achieve four key objectives: 1) to investigate the historical and cultural significance of tuna in the diets of Mediterranean coastal populations, 2) to assess potential differences in pollutant levels in tuna specimens from the pre-industrial and post-industrial eras, 3) to examine adaptive changes—at both genetic and epigenetic levels—in ancient and contemporary Mediterranean bluefin tuna populations, as well as among past and present communities engaged in traditional tuna fishing practices. Our study shows: significant differences in polycyclic aromatic hydrocarbons levels between ancient and modern bluefin tuna vertebrae; evidence of distinct population structures between ancient and contemporary tuna specimens; potential adaptive responses of tuna to anthropogenic pressures; potential variations in the allele frequencies of detoxification of pollutants-related genes between populations that historically consume tuna fish and those that do not. By integrating historical, genetic, and ecological perspectives, this research will provide a comprehensive understanding of the long-term interactions between Atlantic bluefin tuna and human populations in the Mediterranean area. Our findings will not only shed light on the extent of human influence on marine ecosystems but also reveal the adaptive responses of both species to environmental and anthropogenic pressures. Ultimately, this study underscores the need for sustainable fisheries management and conservation strategies, ensuring the resilience of bluefin tuna populations and the preservation of traditional coastal livelihoods against ongoing environmental change.

Rewriting coexistence: anthropogenic pressures and decline of native freshwater fishes in Sardinia

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Freshwater fishes are among the most threatened vertebrates due to long-term anthropogenic impacts such as habitat degradation, alteration of flow regimes, and the spread of non-native species. These pressures, exacerbated by climate change, have caused significant range contractions and, in some cases, local extinctions of native freshwater fish populations.

In Sardinia, only 7 out of 22 recorded freshwater fish species are native. Among them, *Anguilla anguilla* (CR), *Salmo ghigii* (CR), and *Alosa fallax* (VU) are currently threatened and at high conservation risk. By analyzing historical distribution data from 802 sampling sites across Sardinia over a long time period (1940–2022), we observed significant habitat range contractions for native fish species. In particular, the most pronounced declines were recorded for the Mediterranean trout (*S. ghigii*) and the European eel (*A. anguilla*), which showed decreases in occurrence of 53% and 50%, respectively.

The twaite shad (*A. fallax*), once widely distributed in Sardinia's major rivers, is now detected in only 1% of surveyed sites. Its populations are mostly landlocked in reservoirs due to river fragmentation caused by barrier construction. Although isolation has in some cases shielded native Mediterranean trout from genetic introgression due to non-native trout stocking, it has also heightened their susceptibility to inbreeding and extreme floods and drougth events. Genetic analyses on the Mediterranean trout revealed the presence of non-introgressed populations, only in some isolated mountain streams.

These results highlight the effect of long-term impact of human activity on insular freshwater ecosystems. In this contetext, the urgent need for conservation measures, in line with the EU Habitats Directive (92/43/EEC), is evident.

Human and animal evolution, the strange case of feralization in the Sardinian pig

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Animal domestication has significantly transformed Earth's biosphere promoting the artificial selection that has changed the animals evolutionary trajectories. In addition to this intertwining story, many domestic animals returned to the wild due to human intentional actions or unintentional episodes leading to feralization and to feral animals often showing wild features as a consequence of new and intriguing evolutionary processes. Despite its relevance, study of feralization is still relatively neglected. Feralization arises in many animal populations however *Sus scrofa* is an optimal model to delve deeper into this process. We investigated the evolutionary trajectories followed by feral swine in Sardinia, affected by resultant of natural selection and traditional husbandry (artificial selection), genetic drift and gene flow with sympatric wild boar. Using an interdisciplinary approach, we collected historical, genetic and phenotypic traits and addressed them as co-evolved elements. To get successfully the research goal, we grappled with characterization of swine populations' genetic structure using genome approaches. Using over 3,000 Single Nucleotide Polymorphism we found sign of selection in specific genomic regions including genes linked to litter size, sense of smell and number of teats. The screening of genomic variability was useful to highlight the effect of a peculiar artificial selection that modulates its weightiness due to the concomitant action of natural selection. In particular, results suggested that Sardinian cultural diversity in pig husbandry acts pushing down gene flow towards wild boar while favoring adaptations to life in the wild, creating a unique genetic pattern in feral pigs, different both from the domestic and the wild genetic makeup.

Our contribution opens a discussion on topics of global interest in Anthropocene environments and for rewinding Europe program: the preservation of cultural heritage linked to human traditions and related impacts on biodiversity, agriculture, economy, human and animal health.

Neanderthals versus Sapiens in Southern Italy: the reconstruction of dietary habits inferred from zooarchaeological and paleogenome data

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In the last twenty years detailed zooarchaeological analyses on faunal assemblages from different sites in southern Italy referable to both Mousterian and Upper Palaeolithic technocomplexes (MIS 4-3), giving us the possibility of identifying differences in animal exploitation between Neanderthal and AMH populations. In Apulia, it was possible to highlight a specific treatment of ungulate carcasses by Neanderthals, not recorded in Sapiens assemblages

During the Late Mousterian, this region was characterized by frequent and prolonged drought climate conditions that could have caused the scarcity of vegetable foods and, consequently, a lack of important nutritional compounds. Neanderthals may have responded to this crisis by increasing the exploitation of ungulates: bone grease rendering was likely one of the dominant activities conducted on-sites. Considering the ethnographic data related to the economic subsistence patterns

of hunter-gatherers, we merged zooarchaeological and palaeoecological data with the nutritional value of the anatomical parts in medium-large ungulate carcasses. Additionally, a paleo genome analysis was carried out to investigate possible genetic bases of the differences in fat metabolism between Neanderthals and Sapiens. Our results show a low daily energy intake from carbohydrates for Late Mousterian populations in Southern Italy, in contrast to a surplus of animal protein and fats. In addition, analysis of the FADS genes involved in the LC-PUFA biosynthesis, underline the importance for these human groups of 18-carbon unsaturated fatty acid intake from animal resources, to address the low availability of these compounds from plant resources. The acquisition of these data opens up interesting new scenarios on possible differences between Neanderthals and Sapiens both in their possible metabolic processes and in the adoption of different techniques in food processing. In particular, the gluconeogenesis and external fermentation of food are discussed.

Settlement dynamics and memory of places in the SUs 13-11 of the Oscurusciuto rock shelter

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The Oscurusciuto rock shelter opens on the right side of the ravine of Ginosa (Taranto, southern Italy). Its stratigraphic sequence (at least 10 occupation layers) covers a time span between MIS 4 and about 43-42 ky ago, providing relevant information on the settlement dynamics, behavior and economy of the late Neanderthal groups and contributing to make Oscurusciuto an international key site. This study focuses on the evolution of the camp organization, as shown by palimpsest stratigraphic units (SUs) 13 and 11 (about 55 ky ago), with the aim of reconstructing the settlement dynamics and the long-lasting processes that shaped the site structure over time. From a methodological standpoint, an interdisciplinary approach was applied, based on the central role of spatial archaeology. Data from hearth thin sections, sedimentology, Raw Material Units (RMUs), lithic technology, zooarchaeology were analyzed from a geomatic perspective to shed light on the formation processes of the contexts, as well as the preservation and functional interconnection between the activity areas. To this end, a three-step protocol was designed, which includes: 1) characterization and selection of relevant data, 2) taphonomic, and 3) functional analysis of the contexts.

The results of the study indicate the formation of SUs 13-11 in a low energy setting, with displacement processes of the materials that are primarily attributable to human activity (e.g., entropy of the camps, voluntary/not voluntary removal of materials). The palimpsest effect represents the main factor affecting the high-resolution visibility of the site spatial organization. SU 13 is a short-term palimpsest with a good archaeological visibility of the settlement organization, while SU 11 is a long-term palimpsest analytically dissected into two sub-units (also coherent from a sedimentological point of view). Despite the limited extension of the studied area, significant results allow us to recognize the long-lasting permanence of a recurrent settlement pattern along the stratigraphic series of SUs 13 and 11, made (mainly) by a succession of long-term occupations, with a gradual accumulation of changes.

These results suggest that the archaeological record reflects the dialectical relationship between long-lasting processes and the historical memory of places. The latter, from a cognitive perspective, may have acted as a strategic survival factor, maintaining the Group cohesion and knowledge of critical resources.

Reading the past through insects: entomological insights from three Italian archaeological sites

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Archaeoentomology is a discipline that studies insects and other arthropods from archaeological contexts, contributing to the reconstruction of past events, climatic and environmental changes, dietary habits, and—particularly in burial contexts—funerary practices, season of death, and the hygienic and health conditions of individuals.

In this work we present three Italian case studies that show the variety and richness of information that insects can provide in archaeological investigations.

Entomological investigations follow a standardized protocol aimed at preserving both the archaeological context and the integrity of biological remains. Field sampling was carried out in three Italian archaeological sites using tools allowing for the recovery of insect fragments and associated materials without causing damages.

The sediment was oven-dried and sieved to facilitate the isolation of entomological specimens from the matrix.

All specimens and fragments were then selected and examined under a stereomicroscope for taxonomic identification verified through comparison with reference collections.

The first case concerns three mummified hermits preserved at the Sanctuary of Madonna della Corona (Verona), where entomological analyses contributed to reconstructing funerary practices and confirming the authenticity of the liturgical vestments worn by the individuals. The second study focuses on the hypogeal crypts of the Pieve di San Cristoforo in Pian di Marte (Perugia), which include drainage channels—sealed in the 19th century—that once connected the burial environment to the outside. These channels allowed the entry of insect species unrelated to the decomposition of buried bodies but valuable for reconstructing the historical ecology of the surrounding area. The third case involves the discovery of a World War I soldier in the Venetian Prealps, for whom entomological analysis provided useful elements to determine the season of death and reconstruct peri-mortem events.

In all cases, Diptera and Coleoptera emerged as the most representative and informative insects, confirming their central role in entomological analyses of forensic, funerary and archaeological contexts.

These case studies demonstrate that archaeoentomology, through a rigorous and multidisciplinary approach, can provide essential data not only for the reconstruction of funerary dynamics but also for a broader understanding of the environmental and historical conditions of archaeological sites.

Sessione
Il pianeta condiviso

Poster

Invisible barriers: the european eel between habitat fragmentation and climate change

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The European eel *Anguilla anguilla* (Linnaeus, 1758), a catadromous species currently classified as "Critically Endangered" on the IUCN Red List, represents a paradigmatic case of how anthropogenic impacts can compromise the coexistence between humans and wildlife. The long-standing relationship between humans and eels, once based on traditional fishing practices, has transformed into a survival challenge for the species, caught between local pressures and global threats.

This study investigated the effects of 54 large dams (height >15 m) on Sardinian watercourses, assessing changes in the distribution of *A. anguilla* upstream and downstream of dams and the ecological status of river mouths. Using geospatial tools (QGIS, Google Earth), drone surveys, and direct sampling methods (passive floating traps for juveniles and electrofishing for adults and subadults), historical data (1940-2025) were integrated with recent observations to assess species persistence in the affected basins.

These results highlight a significant local impact: a 75% range contraction in dammed basins and an 85% reduction in presence compared to control sites. Dams function as near-total barriers to juvenile upstream migration, with only 2 out of 54 facilities equipped with functional fish ladders. Drone deployment enabled detection of flow persistence in the reaches immediately downstream of dams, assessment of river mouth conditions, and estimation of river quality through the Fluvial Functionality Index.

Beyond river habitat fragmentation, eels face an additional global threat: climate change, through increasing temperatures and altered ocean current patterns, may compromise reproductive migration to the Sargasso Sea. The European eel case demonstrates how human impact, although localized, is embedded within broader environmental transformation dynamics, altering ancient evolutionary equilibria.

Restoring river connectivity and ensuring the ecological functionality of watercourses must be prioritized to ensure species conservation and enable sustainable coexistence between humans and the environment.

Sessione

Antropologia dello Scheletro e Bioarcheologia

comunicazioni orali

Morphological Variation of the Hip Bone: Integrating Diachronic, Geographic, and Sexual Dimorphism Patterns Through 3D Geometric Morphometrics

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The human pelvis reflects a complex interplay of locomotor, obstetric, and evolutionary pressures—but not all pelvic regions preserve the same signals of biological variation. This study investigates how different parts of the hip bone— the complete coxal bone and the acetabulum—capture variation related to sex, ancestry, geography, function, and diachronic trends. Using 3D geometric morphometrics (GM), we analyzed a diverse sample of modern and prehistoric individuals to explore patterns of morphological diversity and factors that shape them.

Our comparative sample includes modern Italians from Bologna and North American individuals of varying ancestries (European, Hispanic, Native American), as well as Upper Paleolithic individuals from Arene Candide and Dolní Věstonice.

Principal Component Analysis of the coxal bone revealed strong sexual dimorphism as the primary source of variation (18.3% on PC1), with clear separation between male and female morphologies across populations. Archaeological male individuals clustered with modern males, suggesting long-term stability in sex-specific hip morphology despite substantial temporal distance. Population differences in morphology were more pronounced in females, while males remained more morphologically homogeneous across groups. The analysis of variance (ANOVA) confirmed significant differences between sexes (p¡0.001), with more limited significant differences between males among populations.

The acetabulum, however, told a different story: geographic/population variation is the primary explanatory factor (PC1=23.4%) rather than sexual dimorphism. Bologna specimens (males and females) clustered separately from North American groups, suggesting strong population-specific morphology in this feature. Integration of size (form space analysis) amplified the acetabular differences between groups.

These findings suggest that different regions of the hip bone may reflect distinct aspects of human variation: while the complete coxal bone predominantly captures sexual dimorphism, the acetabulum may reflect ancestry, function, and diachronic trends. This pattern has critical implications for sex estimation and body mass in paleoanthropological contexts and suggests different selective pressures affecting these anatomical regions throughout human evolution. Our preliminary results contribute to understanding the complex interplay between function, development, and biological diversity in shaping human hip morphology.

Anthropology of the Eneolithic Site of Colombardo

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Evidence of settlements and necropolises of Eneolithic culture in Sicily is scarce. This work presents the first anthropological data of a settlement and hypogeal necropolis discovered in Contrada Colombardo, in the province of Ragusa. The excavation, conducted in 2015 by the Superintendence of Ragusa, on the occasion of the decommissioning of a large industrial storage facility, brought to light a complex settlement, characterized by three Long Houses and a hypogeal tomb, which constitutes a typological unicum in the funerary landscape for the Copper Age on the Hyblean hills. The tomb is divided into three burial chambers, one of which incomplete and unused. The tombs were found inviolate and sealed by lithic doors. The first chamber housed a single burial (T1: S1), a male individual in right lateral decubitus and in a crouched position. The second chamber hosted a collective burial of 8 individuals (T2: S2-S8). The intensive use of red ochre is visible in both chambers. In chamber 2 there is evidence of depositional rituals, such as the particular arrangement of some bodies in secondary position. The anthropological study highlighted the presence of adult individuals of both sexes and of an infant individual. Anthropological, Archaeometrical, Paleopathological and Isotopical parameters useful for the reconstruction of the biological profile and of the paleoecology of the human group were detected. On the basis of the investigations conducted, it is possible to hypothesize a familiarity between the occupants of the hypogeal complex, the possible cause of death of the individual in T1 and the health status of the group. Morphometric differences support the hypothesis of a population heterogeneity.

Biological and cultural insights from the Eneolithic necropolis of Celletta dei Passeri (Forlì, Italy): proteomic and multi-isotope analyses

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The necropolis of Celletta dei Passeri was excavated between 2009 and 2010 in the city of Forlì (Italy). The site represents the largest and best documented Eneolithic necropolis in Emilia Romagna and one of the most important ones in northern Italy. It encompasses 75 graves, only 51 containing human remains, along with the intentional burial of a dog. As regards grave goods, most individuals are buried with a jug placed at their feet, while some of them are distinguished by the presence of copper weapons (seven adults) and flint arrowheads (19 adults and four non-adults). This study aims to reassess the biological profile of these individuals and to investigate their provenance and dietary habits in order to highlight potential differences that may help to better define their role within the community.

For the reassessment of the biological profile, we strengthen the sex estimation through enamel peptide analysis targeting the amelogenin protein. We were able to analyse all the adult individuals buried with copper weapons, along with some of those associated with flint arrowheads (including all the non-adult individuals and four adults for which at least one tooth was preserved). To investigate provenance, we conducted Sr isotope analysis on 32 individuals, as well as on some faunal remains from the site, including the dog. Finally, 13 individuals – both armed and non-armed – were subjected to C and N isotope analysis to reconstruct their diet.

Amelogenin peptide analysis confirmed that two of the adult individuals with copper weapons are female, as previously suggested by anthropological analysis. These represent the first documented cases of armed female burials in Eneolithic Italy, challenging traditional assumptions about gender roles in prehistoric societies. As regards provenance, the vast majority of the analysed individuals appear to be local, with the sole exception of a young adult male buried with copper weapons. In addition, some faunal remains – including the dog – are non-local, raising new questions about transhumance and trade networks. As for diet, no major differences were observed, being the results consistent with a C3 plant-based diet, typical of the period.

In conclusion, the multidisciplinary analysis of Celletta dei Passeri combined biological and cultural data, providing new insights into the complexity of Eneolithic funerary practices and social dynamics.

Human remains from the Monte Claro site of Acquacadda (Nuxis, Italy): archaeothanatology and implications for funerary activities at the cave.

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Caves have always been of interest to humans. As well as providing shelter and dwelling places, their suggestive nature made them an ideal choice for religious and burial practices. Sardinia has an established tradition of cave occupation, dating back to the island's first colonisation. The ritual and funerary use of caves is attested from the late Neolithic, persisted throughout prehistory, and reached its peak during the Copper Age, with the Monte Claro culture (2700-2200 BCE). These people practiced a great variety of burial practices, with cave burials being a prominent feature of southwestern Sardinia. The study of this aspect has been limited by the complex nature of cave diagenesis but has recently gained momentum. The site Acquacadda (Nuxis – SU, Italy) is a karstic cave located adjacent to the copper mine of 'Su Montixeddu' and was used as a dumping site for mining debris. The archaeological significance of the site was well-known for decades, but only in 2019 the University of Cagliari initiated a formal excavation. Five campaigns have revealed the presence of an impressive expanse of pottery fragments of various sizes and types, all belonging to the Monte Claro culture. This 'pavement' is likely linked to ritual practices of great importance. In addition, human remains have been found in almost all the areas investigated, scattered and with no anatomical connection preserved. Through archaeothanatology, this study contributes to the investigation of the processes that led to the formation of the assemblage, and the funerary practices that took place inside the cave. We conducted the mapping of the skeletal elements through GIS along the anthropological study of the bones. Based on 1074 human bones that were identified with different degrees of preservation and fragmentation, a MNI of 14 individuals (7 adults and 7 sub-adults) was calculated. All skeletal districts are represented, suggesting that the assemblage derives from disturbed primary burials, whose original position is unknown. The scattered dispersal of the bones in relation to the ritual pavement suggests that the agent of disturbance is water, which recurrently flooded the cave. Future campaigns will reveal the extent of the anthropogenic use of the cave and the primary position of the inhumations. Acquacadda is to date the largest cave site of the Sardinian Copper Age, and its exploration will be fundamental to our understanding of the ritual behaviors of the Monte Claro people.

Exploring population dynamics through phenotypic diversity: A Gower-Based Analysis of the Terramare necropolises

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This study investigates the biological relationships of Bronze Age populations associated with the Terramare culture in Northern Italy through a comprehensive biodistance approach. Recent research has highlighted the value of integrating diverse phenotypic variables to better understand past population dynamics. In line with this, the present analysis combines metric and non-metric cranial and dental traits, which are non-destructive, macroscopically observable, and biologically informative.

The Gower distance coefficient - well-suited for datasets that include both continuous and categorical variables - was applied to five analytical scenarios, each of which incorporated an expanding set of comparative populations from Europe and beyond (comprising Eneolithic and Late Bronze Age Western Romania, Classical Chersonesos, Late Neolithic to Early Iron Age Southwest Germany, and Roman-period Lebanon). Principal Coordinates Analyses (PCoAs) were used to visualise inter- and intra-population relationships and to assess how the inclusion of additional groups affected clustering patterns. The results consistently show increasing internal cohesion among individuals from the Terramare necropolises, suggesting shared ancestry or prolonged biological interaction.

Notably, individuals from Olmo di Nogara - the earliest site in the Terramare sample - frequently occupied intermediate positions in the multivariate space, indicating its potential role in the demographic structuring of the Terramare populations. Some patterns also suggest biological affinities with Eneolithic populations from Western Romania, pointing to deeper historical connections that may reflect broader migratory processes across Europe during the second millennium BCE.

The patterns revealed through this analysis emphasise the complexity of the Terramare populations. To validate and expand upon these findings, our current research incorporate targeted ancient DNA and isotopic studies, particularly focusing on individuals exhibiting unexpected clustering - especially females - who may represent non-local individuals and pathways of mobility within the Terramare network.

Of Graves and grains: Isotopic Signatures of Social Differentiation at Novilara (Central Italy, 8th-7th c. BCE)

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The Early Iron Age in Italy represents a key moment in the formation of pre-Roman cultural identities, marked by the emergence of distinct groups such as the Latins, Villanovans, or Picenes. This cultural diversification was accompanied by the development of increasingly differentiated social structures, as reflected by funerary variability. However, the biological implications of these inequalities - and how they shaped individual life histories - remain largely unexplored. In this context, dietary analysis offers a valuable approach to test whether differential access to resources can serve as a proxy for social stratification. This study investigates carbon (^{13}C), nitrogen (^{1}N), and sulfur (^{3}S) stable isotopes from bone collagen at Novilara (Marche region, 8th–7th century BCE), one of the largest and most significant necropolises of this period. Culturally linked to the Picene of Central-Eastern Italy, the site exhibits marked variability in grave goods, suggesting a society structured around distinct roles and unequal access to economic resources. To reconstruct dietary habits, we analyze bone collagen from 148 individuals (37 males, 73 females, 12 unsexed adults, and 26 non-adults), alongside four faunal samples.

Isotopic data are compared by sex, funerary treatment (type and number of grave goods), and skeletal and dental proxies of developmental stress (stature, enamel hypoplasia, cribra orbitalia). Results indicate a primarily terrestrial diet based on C plant products and variable animal protein input. Statistically significant differences in ^{13}C and ^{1}N values separate the sexes, with males showing higher ^{1}N and ^{13}C values than females (mean differences = 0.4‰ and 0.2‰, respectively). Among adults, ^{1}N values correlate positively with stature, and in males, individuals buried with weapons tend to exhibit higher ^{1}N ratios, although these trends are not statistically significant. No specific patterns characterize ^{3}S values, but outliers suggest the presence of nonlocals, possibly from the hinterland.

The results suggest that socio-economic status may have influenced access to food resources for a limited subset of individuals within the community. Conversely, the primary axis of dietary differentiation appears to be structured according to sex criteria. These results highlight the value of stable isotope analysis in reconstructing subtle forms of social differentiation that may not be fully captured by material culture alone.

Bodies at work: bioanthropological evidence of occupational stress in the Baratti necropolis (4th–3rd Century B.C.)

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The study of skeletal changes due to functional activity has long been a central topic in anthropological research. Despite methodological limitations associated with the assessment of specific indicators, widely discussed in the scientific literature, the integrated analysis of various biomechanical and functional stress markers allows for the development of interpretive models to reconstruct the daily activities and living conditions of past populations.

This study examines burials from the Baratti necropolis (Populonia, Livorno), dating back to the 4th - 3rd century B.C. The site comprises over 150 tombs discovered along the beach. Archaeological evidence suggests that this funerary area was predominantly utilized by individuals engaged in local port and metallurgical activities. To date, the bioanthropological analysis has focused on 65 individuals of both sexes and all age groups.

In addition to reconstructing the demographic profile, the study examined skeletal indicators useful for detecting adaptive modifications: stature, development of muscle attachments, traumatic injuries, joint degeneration, cranial porosities, and lesions attributable to physical activity.

The results reveal clear signs of a selected population: the low presence of children and the predominance of males (more than twice the number of females) suggest a community with a strong labor-related character.

Trauma is prevalent: over half of individuals have bone fractures (F: 60%, M: 52.4%) and more than 70% show signs of repeated microtrauma. Joint diseases are common, especially in young men (60% ages 20–35, 88.9% ages 36–45). Around 33% of vertebrae in both sexes exhibit spondyloarthrosis, mainly in the thoracic and lumbar regions, with notable sex-based differences. Muscular attachment development suggests intense physical activity from a young age in both genders.

Skeletal evidence from Baratti shows a sample population engaged in physically demanding labor. Biomechanical stress markers, high trauma rates, degenerative conditions, and the biological profile of the sample suggest a group involved in labor-intensive and hazardous tasks with exposure to occupational risks.

This study highlights the relationship between labor, the human body, and social structure, providing substantial information about the living conditions of workers during the Etrusco-Roman period at Populonia.

A Bioarchaeological Study of Burial Unit 1 from the Church of Santa Maria Maggiore in Vercelli

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1. Context and aim of the study: Situated in Vercelli, in Piedmont, the Church of Santa Maria Maggiore was built in the 18th century and includes an underground burial area conceived in tandem with the main church structure. This expansive subterranean cemetery was a burial place for local clergy and nobility members until the mid-1800s. Burial Unit 1, the first tomb encountered upon entering the space, was intended for a single occupant. Set against the wall, the burial consists of a brick-built trapezoidal structure housing a wooden coffin. Within lies a mummified individual in primary deposition, remarkably well preserved and still dressed in garments that remain in excellent condition.
2. Methods and Materials: This study presents the anthropological and paleopathological analyses of the individual, carried out through CT imaging and 3D visualizations to construct a biological profile and assess skeletal diseases and trauma. The investigation was further complemented by histological examinations, entomological studies, historical costume analysis, and gas chromatography performed on medical gauze wrapped around the individual's head.
3. Results: The mummy belonged to a middle-aged male, with an estimated stature of 173 cm, who exhibited multiple pathological conditions. These included dysplasia of the left hip with advanced osteoarthritis, as well as generalized degenerative changes affecting the vertebrae, shoulders, and knees. CT imaging also revealed multiple vascular calcifications in the heart and aorta, consistent with arteriosclerosis. Histological analysis showed a thick keratinized layer corresponding to the outermost part of the epidermis, with no preservation of the underlying Malpighian epithelium or other soft tissues. Entomological investigation identified insect fauna characteristic of underground burial environments, indicating that the body had not been exposed before interment. The study of the clothing confirmed that the garments dated to the second half of the 18th century and were consistent with the attire worn by a man of high social rank.
4. Conclusion: The multidisciplinary investigation of Burial Unit 1 from the Church of Santa Maria Maggiore in Vercelli provides valuable insights into the life, death, and health of an 18th-century high-status individual. The study highlights the significance of combining advanced imaging, laboratory analyses, and historical material culture to reconstruct past lives.

Transformed by fire, a ritual practice dating back to the Early Neolithic in Italy. Interdisciplinary analysis of burnt bone remains in Lugo di Grezzana (Veneto), 5000-4850 cal BCE

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The use of fire for the treatment of human remains in funerary rituals characterises Italian prehistory since the Neolithic, being the dominant funerary practice from the Late Bronze Age to the Early Roman period, with regional differences. New osteoarchaeological and radiometric data allow us to confirm the occasional use of fire as a transformative element for the body already in the Early Neolithic. During the excavation of the Early Neolithic settlement of Lugo di Grezzana in 2003 and 2005 (Fiorano Culture – province of Verona, Veneto, northeastern Italy), five pits were discovered, each containing burnt osteological remains. One pit, interpreted as an oven (ES 541 sector XVI), yielded numerous bones intermingled with abundant fragments of pottery and flint. Most of the bones were identified as non-human remains. However, the morphological examination revealed fragments of diaphyses with characteristics consistent with human bone. Subsequent histomorphological analysis confirmed the taxonomic identification of these fragments as belonging to the genus Homo and provided insights into the age distribution, indicating that they belonged to individuals spanning different age groups.

The 14C analysis of charcoal and bones suggests that the use of ovens dates from 5400 to 5000 cal BCE. A new radiocarbon date of a calcined human bone fragment has been placed between 5024 and 4845 cal BCE, indicating that the oven was likely reused as funerary structure during the final phase of the site use. This discovery has increased the number of recent findings of burnt human bones within Neolithic contexts in Italy, prompting us to reflect on the significance of their presence as possible evidence of the early evidence of fire rituals involving the treatment of human remains in the Italian Peninsula.

A microscopic analysis of cremated bone microstructures preservation and age-at-death estimation in an Imperial Roman community in central Italy

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Cremation was a widespread funerary practice in antiquity, carrying symbolic and cultural significance associated with the treatment of the body and the transition to the afterlife. Nevertheless, the elevated temperatures involved in the cremation process induce substantial modifications and alterations in skeletal and dental remains, thereby limiting the ability to reconstruct the biological profile of ancient cremated individuals.

The present study focuses on the potential of histological and histomorphometric analyses applied to a sample of 18 long bone fragments (humeri and femora), pertaining to different cremations, from the Imperial Roman necropolis of La Cona (Teramo, Abruzzo, 1st century BCE - 1st century CE), which is distinguished by a high number of cremations.

The bone fragments have undergone standard histological procedures, which involve embedding in epoxy resin, cutting with a diamond blade low-speed saw, grinding to a thickness of approximately 100 µm, and polishing with alumina powder. Thin sections were examined under transmitted light microscopy to assess the degree of preservation of the cortical microstructures and determine any changes related to bone remodelling and ageing process. The age at death was estimated using the osteon population density (OPD) parameter; while secondary osteons, Haversian canal areas and osteon circularity index were collected for each specimen.

Results show a good preservation of the bone microstructures, despite the substantial macroscopic alterations. A high inter-individual variability in OPD was observed, which aligns with the macroscopic estimation of the age at death. As the body undergoes the process of ageing, secondary osteons and Haversian canals tend to decrease in their dimensions and become more circular. The observed trends reveal a positive correlation between biological age, bone remodelling, and histomorphometric features. This study confirms the reliability of microscopic bone analyses in elucidating the biological and ageing dynamics of human bones, highlighting its value in the investigation of ancient cremations. This study enabled the refinement of the demographic profile of the cremated population of La Cona and the acquisition of significant information regarding the cremation ritual in this population during the Roman period.

Taphonomy and archaeothanatology reveal details of the different mortuary programs in collective burials from prehistoric and protohistoric Sardinia

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The practice of collective burial is a widespread phenomenon in prehistory and protohistory. Conceptually, it has been proposed that the commingling of skeletal remains may reflect the societal value of group identity and the construction of an ancestral collectivity. Themes of separation, decomposition, and reintegration of human remains are attested in ethnographic studies and have been proposed as part of the mortuary program for various archaeological settings. However, specific mortuary gestures and phases of manipulation can vary widely within this broader categorization, reflecting ritual, cultural, and social differences – including early forms of stratification – despite a collective funerary presentation. Applying archaeothanatological and taphonomic principles to human remains can help identify these gestures, such as defleshing, secondary deposition, or repeated disturbance of primary depositions, thus revealing distinct funerary programs.

We analyzed collective burials from different Sardinian cultural contexts spanning the Neolithic to the Bronze Age. Results suggest that commingled assemblages from Giants' Tombs (e.g. Muttas Nieddas, Gesico) result from continuous disturbances of primary depositions, given the finding of skeletal elements from labile joints (e.g. carpals, phalanges, hyoid bones). Conversely, funerary sites in caves from the Late Bronze Age (e.g. Lu Maccioni-Alghero and Grotta Tueri-Perdasdefogu) have yielded cutmarks compatible with defleshing on the skull and long bones, suggesting that decomposition happened outside, and that selected elements were carried in the cave, performing a cleaning action when necessary. A similar practice may apply to the Late Neolithic domus de janas of San Benedetto (Iglesias), where remains appear selected, mostly comprising crania and long bones. However, evidence for cut marks is inconclusive due to taphonomic degradation. At Bingia 'e Monti, a hypogeic-megalithic tomb built in the mid-late Copper Age and with a complex history of reusing in the initial Bronze Age, primary depositions and their later disturbances are apparent from excavation pictures. Strikingly, we discovered one instance of cut marks in a frontal bone from lower layers, hinting to a succession of different funerary programs at the site. This ongoing study offers skeletal evidence aiding the reconstruction of multi-stage mortuary practices and to the detecting cultural changes in socially-mediated funerary representations.

Osteobiography of the people from Nora. Life and death at the Phoenician and Punic Nora (7th to the 2nd cent. BC): A bioarchaeological and multianalytic approach to organic evidence from the western necropolis

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The archaeological site of Nora, located in southern Sardinia (Cagliari), serves as a pivotal case study for the reconstruction of population dynamics, bio-cultural exchange, and individual mobility within the Mediterranean societies of the 1st millennium BC.

Excavations conducted by the University of Padua in the western Phoenician and Punic necropolis have revealed a stratified funerary record, spanning from the 7th to the 2nd cent. BC, including single secondary cremations and both primary and secondary inhumations. This diachronic sequence provides critical insight not only into the evolution of mortuary practices, but also into the biology of the people living at Nora during the transition from the Phoenician to the Punic sociocultural framework.

This contribution presents the results of a multi-proxy bioarchaeological study that integrates human osteology, histology, biogeochemistry and proteomics, archaeozoology and archaeobotany aiming to reconstruct the individual osteobiographies, funerary customs and asses their variability in relation to biological parameters, rituals structures, and mechanisms of social inclusion and exclusion.

Our results emphasise a dynamic spectrum of funerary and ritual behaviours, reflecting evolving cultural frameworks and social structures over time. Practices such as the selective inclusion of individuals, age-differentiated treatment—especially of subadults—and variability in the use of faunal deposits indicate the presence of structured ritual grammars and evolving conceptions of the body and personhood. The exceptional recovery of textile fragments associated with cremated remains offers rare insight into post-cremation treatment and the symbolic role of perishable materials within the funerary rite.

Archaeozoological and archaeobotanical evidence further clarify the operational sequences underlying these practices, revealing nuanced links between organic offerings and the biological and social identity of the deceased. As such, Nora provides a methodologically rich context for examining biocultural interactions, mobility patterns, and identity formation in a culturally-mixed setting, advancing the interpretive potential of bioarchaeology within the Phoenician and Punic studies.

Conflict, Plague, and Burial: Multi-Proxy Evidence from a Medieval Urban Cemetery of Verona

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This study provides a multi-proxy investigation of an urban cemetery in Verona, northern Italy, offering new insights into infectious diseases, healthcare practices, and funerary behaviour during the late medieval period. Focusing on the Arditi d' Italia Square cemetery, which contains approximately 800 burials, the research presents the results of bioarchaeological analyses conducted on selected individuals interred in double and single burials and mass graves. New radiocarbon dates indicate that part of the cemetery was used between the 12th and 14th centuries, a period characterised by a critical political and military phase in Verona's history that overlaps with the outbreak of the Black Death. By combining anthropological, archaeothanatological, paleopathological, aDNA, and isotopic analyses, this research aims to explore how the local community reacted to these periods of crisis and confronted death. Due to the complexity and extent of this cemetery, 54 burials containing 102 individuals were selected for this study. The preliminary results show that mass graves were used between the 12th and 13th centuries, while most single and double graves were interred in the 14th and later centuries. The unusual position of the bodies buried within the mass graves and the irregular pit morphology suggest that the individuals were laid down in haste. Conversely, the individuals buried in the double burials were positioned with care, with some embracing each other. The individuals buried in the mass graves were mostly men, whereas the remaining burials showed a homogeneous distribution of male and female individuals, with a slight prevalence of females and children. Carbon and nitrogen isotope analysis revealed differences in diet between the individuals. Those buried in the 12th-13th-century mass graves had a diet based on the consumption of C4 plants, whereas the other individuals, buried in the 14th century, had a diet based on C3 plants. This extraordinary context offers an opportunity to investigate the intertwined impacts of conflict and epidemics on late medieval Italian urban communities.

Directional Limb Asymmetries and Lifestyle: A Diachronic Analysis of Three Pre-Industrial Populations from the Abruzzo Region (Central Italy)

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Humans are known to exhibit a certain degree of limb asymmetry, partly due to genotypic and phenotypic variability (fluctuating asymmetry), but more significantly as a result of lifestyle and preferential limb use over the course of life (directional asymmetry). Previous studies have reported a general population-level pattern of right-sided upper limb and left-sided lower limb dominance, although this may vary according to daily activities. This study aims to evaluate the degree of directional asymmetry in three populations from Abruzzo (central Italy), spanning different historical periods and subsistence strategies, in order to assess changes in the degree and pattern of asymmetry over time and in relation to lifestyle. Thirty sagittal and transverse dimensions were recorded from paired humeri, radii, ulnae, femora, tibiae, and fibulae in adult individuals from Opi – Val Fondillo (Bronze Age, hunter-gatherers, 59 individuals), Sulmona – Contrada Santa Lucia (Classical Age, quarry workers, 49 individuals), and Teramo – Sant'Anna (Medieval Age, craftsmen, 38 individuals). Directional asymmetry (%DA) was calculated for each measurement and statistically compared both within and between populations. Population-level handedness was also assessed. As expected, greater asymmetry favoring the right upper and left lower limbs was observed in the more ancient populations with more physically demanding lifestyles. Statistically significant asymmetries were found not only in transverse dimensions (related to muscular development) but also in sagittal ones, suggesting that intense physical activity began during skeletal development. In contrast, the more recent and sedentary Teramo population showed lower asymmetry (limited to right-side dominance) and a largely symmetrical skeleton in 18 out of 30 measurements. These findings indicate that right upper limb dominance remained consistent over time and across subsistence strategies, whereas left lower limb dominance appears more influenced by lifestyle and environmental factors. Overall, the observed reduction in limb asymmetry over time reflects broader changes in lifestyle and physical labor, particularly the shift from natural/rural to urban environments and from dynamic, labor-intensive activities to more sedentary and evenly distributed ones.

Keywords: Directional asymmetry, limb dominance, handedness, Abruzzo, skeletal anthropology, lifestyle

New Perspectives on Neolithic Lifeways in Northeastern Italy: Stable Isotope Evidence from Arco and Riva del Garda (Trento)

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Following the transition to a Neolithic lifestyle, the consolidation of agricultural and pastoral practices is reflected in the 'Middle Neolithic' cultures, represented in northern Italy by the Square Mouthed Pottery (SMP) Culture. This phenomenon lasted throughout the 5th millennium BCE and is characterized by both cultural uniformity and adaptability to diverse geographical environments. Archaeobotanical and archaeozoological evidence indicates varied subsistence strategies, including agriculture, livestock breeding, and pastoralism. However, limited information is available on human adaptations in such contexts, particularly in northeastern Italy. Recent excavations in the Upper Lake Garda area have uncovered new cemeteries of the Square Mouthed Pottery Culture at Arco – Via Degasperi (14 burials) and Riva del Garda – Via Brione (10 burials), located about 5 km apart. Most of the individuals were placed in stone cist graves, lying on their left side and facing east. By combining archaeology, anthropology and stable isotope analysis, this study investigates dietary practices using carbon, nitrogen, and sulphur isotopes in bone collagen from 24 humans and 8 domesticated animals. Additionally, 9 permanent teeth were also analysed to explore infant feeding practices and early-life physiological stress. Animals from Trentino show high carbon values, possibly reflecting pasturing in open environments, while human samples exhibit more negative carbon values. This suggests non-alimentary exploitation of the sampled animals. Most humans from Arco and Riva del Garda had a terrestrial diet based on resources from a temperate C3 environment, but higher nitrogen values at Riva del Garda suggest a greater intake of animal protein, with freshwater resources as a possible contributing factor. Sulphur values show no differences between humans and fauna, indicating a local origin for most individuals, except for one potential non-local case, an individual associated with distinctive grave goods. Comparisons between Trentino and other sites in Liguria, Emilia-Romagna, and Veneto reveal distinct isotopic patterns within the SMP horizon. This study confirms interregional variability and highlights a striking intra-regional variability in human resource exploitation and adaptative strategies, contributing to a deeper understanding of Neolithic lifeways in northern Italy.

Reconstructing Bronze Age Diets in the Po Valley: Bone and Dentine Isotope Evidence

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This research investigates local ecological dynamics, subsistence models, and differences in access to food resources, following the ecological hypotheses developed within the PRIN 2022 SHOVELING project. A central focus is the notably high prevalence of shovel-shaped incisors among Terramare sites in Bronze Age Northern Italy. This study aims to determine whether individuals with and without this dental trait show distinct dietary patterns, potentially reflecting different cultural or biological backgrounds. To explore this, stable isotope analysis of bone and dentine collagen is employed.

We analyzed 344 humans and 20 terrestrial animals of both domestic and wild species from four Terramare necropolises in the Veneto region: Castello del Tartaro, Bovolone, Franzine Nuove, and Olmo di Nogara. Faunal remains provide a local terrestrial isotopic baseline representative of a diet mainly based on C plants. Compared to this baseline, results show that human ^{13}C and ^{1}N values are higher than those of animals, indicating regular consumption of animal proteins and C plants, such as millet. Overall, the Terramare communities show relatively homogeneous isotopic values, suggesting a shared diet and broadly equal access to resources, as observed at sites like Olmo di Nogara. However, some sites, such as Bovolone, show greater variability, likely reflecting sex-based dietary differences, with more variation among males. No significant isotopic differences were observed between individuals with and without shovel-shaped incisors. Based on current data, no distinct dietary practices can be associated with this trait.

Incremental dentine analysis was carried out on 20 first molars from individuals already sampled for bone collagen. Isotopic profiles (^{13}C, ^{1}N, ^{3}S) trace early-life diet, including breastfeeding and weaning. Preliminary data show similar weaning ages (3–4 years), though isotopic evidence suggests variation in the timing of C plant consumption, with earlier or later intake observed between individuals. These findings highlight the complexity of dietary practices at Terramare sites in Bronze Age Northern Italy. While isotopic homogeneity suggests a broadly shared diet and equitable access to resources, site- and sex-based differences point to localized variability. From a broader perspective, the data reflect shared cultural habits alongside adaptative strategies shaped by ecological and social factors in the Po Valley.

Tracing lives in isotopes: multi-isotope perspectives on diet and mobility in Fermo (Marche, Italy) during the Iron Age

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In recent years, growing interest in dietary habits and mobility in past societies has increasingly drawn the attention of scholars, becoming one of the most discussed topics in archaeological research. This trend has been driven by the development of new and advanced biogeochemical methodologies that have significantly enhanced the study of ancient diet and mobility patterns. In this framework, isotope analysis of archaeological teeth and bones has become a key tool for investigating changes in habitat, animal herding, dietary tendencies and migration patterns in the past.

This paper explores the dietary and mobility patterns at the important Early Iron Age site of Fermo (chronology, Marche, central Italy), through a multi-isotopic analysis (^{13}C, ^{1}N, ^{1}O, and ^{3}S) of 33 human tooth root samples and 30 tooth enamel samples of inhumed individuals, along with some faunal bone samples for recreating the baseline.

Since the first systematic studies, the archaeological site of Fermo – comprising the Misericordia and Mossa necropolises, used from the 9th to the 5th century BCE – has been in a lively scholarly debate. Although situated within the territory traditionally associated with the Picene cultural sphere, both its organizational structure and the burial practices reveal notable affinities with the renowned and intricate Villanovan material culture, along with elements of the Picene tradition itself. The funerary complexity of Fermo has made it one of the most debated Iron Age sites in Italy within the academic community, both in past and recent research.

This study is part of a broader research project and aims, first, to demonstrate how a multi-isotope approach can provide a more detailed understanding of dietary and mobility patterns than a single-isotope approach, while integrating with existing isotope data from previous studies at Fermo. Secondly, it seeks to compare these results with data from other contexts across the Italian peninsula to build a comprehensive scenario of the complex social and political dynamics of Iron Age populations.

Enamel histomorphometry of pre-Mesolithic and Neolithic individuals from al-Khiday (Khartoum, Sudan)

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Since 2005, archaeological excavations at site al-Khiday (Khartoum, Sudan) revealed a multi-stratified cemetery (site 16-D-4, al-Khiday 2) with distinct burial phases dating from the Late Pleistocene to the 2nd century CE. This study advances the analysis of the site's bioarchaeological record, previously reliant on standard anthropological approaches, by introducing new data from dental enamel histomorphometry.

Due to its appositional development and lack of remodeling, dental enamel preserves a high-resolution temporal record of early life. Histomorphometric analysis of enamel microstructures provides key data on infants' growth trajectories and physiological stress, allowing for the reconstruction of individual biological life histories.

Seventeen teeth from pre-Mesolithic and Neolithic individuals from al-Khiday were analysed for enamel histomorphometric parameters, including Daily Secretion Rate (DSR), Crown Formation Time (CFT), and Enamel Extension Rate (EER), as well as the identification and prevalence of Accentuated stress Lines (ALs).

In the pre-Mesolithic subset, deciduous upper canines (n=2) show faster DSRs and shorter CFTs than modern standards (mean: 439 days), with their formation beginning early during pregnancy (18 gestational weeks). Compared with modern references, the first permanent molar has a shorter CFT (832 days) and slower DSR, whereas the second permanent molars (n=12) have longer CFTs (mean: 1365 days). Neolithic first permanent molars (n=2) have shorter CFTs (mean: 799 days) and DSRs consistent with modern human variability. All the adults show at least two ALs, with stress peaks identified in first permanent molars around 9 months and second molars at 45 months.

The results suggest that, compared to modern humans, children in al-Khiday grew faster, in line with evolutionary trends towards slower growth trajectories over time. The shorter CFTs in the first permanent molars reflect fast EERs (mean: 22.8 µm/day), whereas the prolonged formation of pre-Mesolithic second molars may relate to their larger size. The observed peaks in ALs at ˜9 and ˜45 months may correspond to critical developmental transitions such as the weaning process.

This study highlights the potential of enamel histomorphometry to reconstruct growth and health status during infancy in past human populations, which complements traditional bioarchaeological approaches and provides new insights into recent evolutionary trajectories.

Tracing Mobility in the “Celtic” Necropolis of Casalecchio di Reno (BO, early 4th – early 3rd c. BCE) through Strontium Isotopes

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The necropolis of Zona “A” in Casalecchio di Reno (Bologna, Italy) is the earliest known “Celtic” cemetery in the region and is attributed to the Boii, a group traditionally associated with East-Central Europe. The necropolis was in use for about a century, spanning at least four generations. Unlike other coeval Celtic contexts in northern Italy, where grave goods often reveal local influences, the funerary assemblages of Casalecchio are characteristically La Tène. This cultural uniformity suggests strong ties with transalpine Iron Age populations. Both archaeological evidence and ancient written sources support the hypothesis of a southward “Celtic” migration beginning in the early 4th century BCE. However, the extent and nature of this demographic movement remain debated.

To investigate mobility patterns, strontium isotope ratios (Sr/Sr) were analyzed from the dental enamel of early mineralizing teeth of 89 individuals, out of 94 recovered skeletons. The sample includes both sexes and all age groups. Results were compared to local baseline values derived from regional isoscapes. Individuals whose values fell outside this range were classified as non-local.

Given the span of the necropolis, a substantial presence of local individuals was expected. Indeed, 46% of the sample shows isotopic signatures within the established local range (0.7092–0.7087). Another 31.5% falls within a lower range (0.7087–0.7080), possibly still local, considering regional geological variation. The remaining individuals display a wide array of values, suggesting heterogeneous life histories and possibly multiple migration events. Some diverge markedly from the local baseline, possibly reflecting distant origins, while others differ only slightly, suggesting movement from nearby areas. Non-local individuals include both sexes and some subadults.

The strong cultural alignment with continental “Celts” raises questions about the biological background of this population and its interaction with local Etruscan groups. Ongoing analysis of non-metric dental traits will contribute to this discussion.

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The Chemical Memory of Teeth: Insights into Lead Exposure during pregnancy and Infancy

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Teeth serve as retrospective archives of individual biological life histories, preserving information from intrauterine life through early childhood and beyond. While dental microstructures embed information on infants' growth trajectories and physiological stress exposure, the chemical composition of dental mineralized tissues may permanently preserve the signatures of the exposure to toxicant during their formation.
Heavy toxic metals can be readily absorbed by humans through food, air, and water, leading to detrimental health outcomes. Among these metals, Pb is particularly significant due to its widespread presence in (proto)industrial human activities. Although extensive research has investigated lead poisoning throughout human history, limited knowledge exists regarding the incorporation of Pb into mineralized human tissues during gestation and the early postnatal period.
In this study we used time-resolved histology-driven laser ablation ICPMS analysis to measure the variation in [Pb] in human dental enamel from contemporary (exfoliated deciduous teeth, N=15), modern (early 20th century, exfoliated deciduous teeth, N=14) and archaeological (seven Roman Imperial necropolises of Central Italy, permanent first molars, N=32) dental specimens. Results show a decreasing trend of Pb concentrations through time. Roman Imperial individuals are characterized by the highest Pb levels (mean = 4.63, SD = 10.91ppm); early 20th century individuals show moderate Pb levels in both prenatal (mean = 3.25, SD = 1.64 ppm) and postnatal (mean = 2.53, SD = 1.86 ppm) enamel, while contemporary children have the lowest Pb concentration (mean = 0.11, SD = 0.2 ppm). The Roman Imperial samples show a very high variability intra and inter necropolises, as confirmed by the high coefficient of variation (CV = 2.36). Peaks in [Pb] are visible before and around birth, probably linked to the remobilization of Ca from the maternal skeleton at the end of pregnancy.
This research underscores the importance of gaining deeper insight into the mechanisms by which lead is embedded in dental microstructures during odontogenesis, and it offers new opportunities for reconstructing historical exposure to toxic metals.

Refining adult age-at-death estimation through semi-automated tooth cementum annulation (TCA) counting

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The accurate determination of age at death is a fundamental aspect of biological anthropology and particularly important on bioarchaeology. This is because it is crucial for the reconstruction of individual life histories and the demographic profiles of past populations. In subadult individuals, maturation stages of skeletal and dental markers, such as tooth formation and eruption and fusion of the ossification centres, are reliable approaches due to the well-defined stages of dental and skeletal development during growth. Conversely, in adult individuals, conventional methods rely on the assessment of degenerative changes, such as dental wear and joint surface modification, or on bone histomorphometry, which are idiosyncratic and influenced by environmental factors.

Among human remains, teeth preserve permanent incremental markers within their microstructure since they don't undergo remodelling. Dental cementum, the mineralized tissue covering the tooth root, continues to form throughout life in rhythmic, annual, layers known as annulations, which are visible in ground sections. These incremental markers offer a tool for a more precise adult age-at-death estimation. However, the process of counting cement annulation is complex and time-consuming.

This study proposes a semi-automated histological analysis of cementum annulations using a proprietary R script to improve accuracy and reproducibility. We analysed 30 permanent canines from the Documented Collection of the Certosa Cemetery (Bologna, Italy, 19th-20th century), selecting specimens based on documented age to explore possible biological variation.

Results indicate a progressive increase in cementum thickness with age, along with a corresponding increase in the number of detectable annulations. However, a slight decline in the precision of exact age-at-death estimation was observed in older individuals, as expected based on previously published work, likely due to the biological decrease of cementum rate deposition over time

By integrating traditional histological techniques with automated image analysis, this approach offers a refined and replicable method for age-at-death estimation. Our results hold significant potential for application in forensic anthropology, bioarchaeology, and palaeoanthropology, contributing to a more accurate reconstruction of mortality profiles in both modern and ancient populations.

Diachronic Variation in Daily Secretion Rates of Human Dental Enamel

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Human dental enamel is a valuable biological record for reconstructing ontogenetic trajectories and physiological stress prevalence in humans. This study investigates the variation in dental enamel daily secretion rates (DSRs, i.e. the daily rate of enamel secretion along the enamel prisms) of first permanent molars in the Roman Imperial period, comparing them with the values available in literature for the same period and for contemporary individuals. Few studies so far have reported a diachronic variation in DSRs: One study suggested a decrease in prenatal DSRs of deciduous incisors between Imperial Roman and contemporary individuals, while another highlighted a diachronic decreasing trend in first permanent molar DSRs in Britain. By examining variations in DSRs, this research aims to investigate possible developmental trends over time.
Fifty-nine first permanent molars dated to Roman Imperial time from the necropolises of Isola Sacra, Centocelle, and Castel di Guido (Rome, Italy), Velia (southern Italy), Civitanova Marche, Urbino, Porto Recanati, and Villarey (central Italy) have been analysed. Histomorphometric assessments on thin sections of the dental crowns allowed quantification of enamel incremental markers to calculate the DSRs.
Results reveal variability in DSRs among Roman Imperial samples. Comparisons with published Roman Imperial and contemporary datasets yield statistically significant differences in mean values, showing a decreasing trend in infants' growth trajectories through time. The lateral inner enamel DSR shows a significant difference among the means of the necropolises, while for the cuspal inner enamel DSR means remain similar.
This research underscores the importance of understanding the regional and temporal variation in enamel growth dynamics and the interplay of biological and environmental factors shaping dental ontogeny. Further studies involving larger datasets from different geographic and chronological horizons are needed to refine interpretations of diachronic changes in human enamel development and their implications for recent human evolution.

The chemistry of birth: rethinking the neonatal line through trace elements LA-ICP-MS profiles

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The integration of high-resolution *in situ* laser-based ICP-MS trace element (TE) analysis with the histological record of dental enamel enables near-weekly resolution of the chemical signatures recorded into the forming tissue. The neonatal line (NNL – the birth marker), present in all the deciduous teeth and often in the first permanent mandibular molar, clearly distinguishes prenatal and postnatal enamel. Its position and histological identification are the reference points for verifying the survival of the individual, as NNL forms between 8 and 14 days after birth, and allows the registration of chemical and/or histomorphological key events along the individual's lifetime.

Synchrotron-based XRF analyses showed distinct shifts in trace element distribution at the neonatal line, with increased Zn concentrations observed in prenatal dentine and enamel across the NNL.

In the present study, conducted on 11 contemporary deciduous teeth with known clinical and dietary history, TE profiles (Sr, Ba, Mg, Zn, and Pb, the latter as a proxy for pollution) were analysed by LA-ICP-MS across the NNL and along the Enamel Dentine Junction.

In most of the cases Zn and Pb show a recurring pattern in proximity of the NNL: a rise of the profile followed by a decline, consistent with a physiological transition associated with the onset of extrauterine life, plausible as metabolic adaptation causing the remobilization of elements from the maternal skeleton to be incorporated in the foetal growing dental tissues. This signal of 'chemical birth' can be misaligned with the histologically observed NNL. The mismatch varies between samples, with an average deviation of about 20 days, which can manifest itself either as an advance or a delay concerning the histological observation.

This discrepancy suggests that chemical and histological signals can reflect distinct and possibly asynchronous physiological processes, while the topographical variation of the enamel thickness along the crown could play a role as well. This study highlights the need for a better understanding of the complex physiology associated with birth, which can be read by histology-driven LA-ICP-MS.

Sessione

Antropologia dello Scheletro e Bioarcheologia

poster

Social Support and Disability after Trauma: an Early Medieval case study from the Villa dei Mosaici di Negrar di Valpolicella (Verona)

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The bioarchaeology of care is an anthropological framework that examines past healthcare behaviors through evidence of disease, trauma, and disability preserved in human skeletal remains. It offers insights into the social structures, cultural values, and caregiving practices of ancient communities, highlighting how societies responded to vulnerability and supported individuals with chronic conditions or impairments. In this study, we applied the bioarchaeology of care approach to the skeletal remains from a single primary burial (tb. 7), uncovered during recent archaeological excavations (2018–2022) at the Villa dei Mosaici di Negrar di Valpolicella (VR, 4th c. AD). The burial, along with the others, is dated to the villa's reoccupation phase (mid-7th c. AD) following its abandonment, and contained the remains of mature adult female who had sustained a severe fracture to the neck of the right femur. Likely caused by a fall, the fracture healed at an unnatural angle, leading to significant changes in bone morphology and structure.
To assess the impact of the trauma and infer aspects of community care, we conducted a biomechanical analysis of the lower limbs. Maximum and biomechanical measurements were taken, and cross-sectional data at midshaft were extracted from 3D models of both femora and tibiae. Femoral asymmetry in torsional moment (J) and total area (TA) was calculated and expressed as absolute, non-directional asymmetry. The right femur shows a 5.25% reduction in TA and 10.25% in J compared to the left, likely reflecting reduced loading on the right limb and compensatory overloading on the left during standing and walking. No such asymmetry is observed in the tibiae.

The trauma, along with secondary structural changes to the right acetabulum and lower lumbar vertebrae, undoubtedly impaired her gait. The lack of effective medical care likely led to a permanent disability, causing intense pain during and after the healing process. Nevertheless, the complete healing of the fracture suggests sustained care and community support, extending even into the post-recovery phase. This is further supported by dietary isotope values, which show no significant differences in her nutritional intake compared to other adults at the site. Notably, however, she is the only individual buried with personal ornaments—three metal Lombard-style armillae still on her forearms—possibly indicating a distinct social status or underlining her uniqueness within the community.

Dental pathologies and dietary patterns in prehistoric Sardinia: analysis of the specimens from Cuccuru Is Arrius (Cabras), San Benedetto (Iglesias), and Santa Caterina di Pittinuri (Cuglieri)

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The spread of farming and the agricultural intensification across Europe are key features of the Neolithic, implying significant transformations in dietary habits, daily practices, and broader lifestyle patterns.

This study aims at understanding dietary patterns and resource exploitation from the Middle Neolithic to the onset of the Copper Age in Sardinia. Data from continental areas suggest a linear trend of agricultural intensification, while environmental conditions in Sardinia may have generated a distinct developmental trajectory of the production economy.

The archaeological record suggests that on the island plant domestication progressed more rapidly in soft-soil, lowland areas near water sources, while other territories remained more dependent on mixed forms of subsistence.

Oral health serves as a key indicator for subsistence strategies, environmental interactions and social organization. It is expected that caries and calculus will increase with greater reliance on cereals as staple resource due to higher sugar intake, whereas a meat-based diet is expected to correlate with greater occlusal wear, alveolar resorption, and antemortem tooth loss due to masticatory stress.

This study tests the scenarios using oral health in three chronologically spanning samples (Cuccuru Is Arrius for Middle Neolithic, San Benedetto for Late Neolithic, and Santa Caterina di Pittinuri for Copper Age).

The examined dental remains include both in alveoli and scattered teeth. The recorded pathologies comprise carious lesions, dental calculus, dental wear, enamel hypoplasia, periapical abscesses,alveolar bone resorption, antemortem tooth loss, and chipping. The results will be contextualized within the framework of previous isotopic analyses, which indicate differential dietary patterns:Cuccuru Is Arrius exhibits evidence of marine resource exploitation, San Benedetto shows a predominance of swine and ruminant consumption, while Santa Caterina di Pittinuri is characterized by a legume-based diet with lower reliance on bush meat.

The analysis conducted so far reveal a slight increase in caries, AMTL, and calculus over time, which is coherent with increased reliance on cereal as a staple food. However, statistical significance is seldom reached, probably due to sample size. Forthcoming analysis will take into account age at death of the individuals under exam, which can significantly bias the results.

Sexual Dimorphism in the Human Cranium: Morphometric Evidence from the Sambaqui Site of Encosta da Lagoa (Brazil)

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Although Homo sapiens shows relatively low sexual dimorphism compared to other species, it still presents notable intraspecific variability, influenced by factors such as diet, ancestry, health, and genetics. Previous research demonstrates that sex estimation methods tend to perform well in population-specific contexts, but their accuracy often decreases in cross-population analyses. Therefore, case studies focused on well-defined populations offer valuable insight into patterns of sexual dimorphism. The Encosta da Lagoa site, located in
Garopaba, Santa Catarina, Brazil, provides a meaningful context for such investigation. Situated 2.2 km inland, the site covers 2,794 m^2 and is associated with the sambaqui tradition, shell-mound funerary
structures typical of coastal hunter-gatherer societies in South America, dated between 7,500 and 1,500 BP.
These communities relied heavily on fishing and shellfish gathering. The site includes a minimum number of individuals (MNI) of 23, including 18 subadults, with both sexes represented. This study aims to explore
sexual dimorphism in this population and to develop preliminary hypotheses about morphological variation patterns. A virtual anthropology approach was applied using geometric morphometrics. Crania of adults were digitized via medical CT scans, with 50 anatomical landmarks and a patch of 1,000 semilandmarks collected.
Comparative data from Indigenous South American individuals housed at the Museo de La Plata (Buenos Aires, Argentina) were also analyzed. Geometric morphometric analysis was performed in both shape and form space. Landmark variation was assessed using Generalized Procrustes Analysis (GPA), followed by Principal Component Analysis (PCA). Classification accuracy was evaluated through Linear Discriminant Analysis (LDA). Results confirm higher accuracy in sex estimation within single populations. The most dimorphic features remain consistent with global findings, with the frontal bone, glabella, mastoid processes, and the entire cranium ranking highest in discriminatory power.

The Roman Conquest and the Epidemiological Transition in Italic Populations of the Abruzzo Region: preliminary data

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The Roman conquest of central-southern Italy marked a turning point not only in the political and cultural landscape of Italic populations, but likely also in their health and disease patterns. This study aims to explore the epidemiological transition potentially associated with the Roman annexation, focusing on skeletal populations from two Abruzzese necropolises: Opi Val Fondillo (4th–5th century BCE, pre-Roman period) and Bazzano (mainly Classical period, Roman era).
A total of 205 individuals from Bazzano and 122 from Opi were examined using standard paleopathological protocols, with particular attention to trauma, degenerative joint diseases, infections, congenital anomalies, and metabolic stress markers. Dental and non-dental alterations were recorded separately for the Opi assemblage.
Preliminary results reveal distinct pathological profiles. In Bazzano, 45 probable non-dental infections (22%), 62 cases of joint disease (30%), 26 traumas (13%), 14 congenital anomalies (7%), 16 metabolic stress markers (8%), and 5 tumors (2%) were identified. In Opi, 70 individuals (57%) presented joint alterations, with 13 traumas (11%), 10 infections (8%), 5 tumors (4%), 8 stress indicators (7%), and 9 congenital anomalies (7%). Dental analysis in Opi revealed 45 cases of caries, 30 antemortem tooth losses, 37 severe wear cases, and 11 periodontal lesions, among other findings.
Compared to Opi, the Bazzano sample shows a higher frequency of non-dental infections (22% vs 8%) and trauma (13% vs 11%), while degenerative joint disease appears more prevalent in Opi (57% vs 30%). These differences may reflect shifting workloads, environmental exposures, and lifestyle changes following Roman integration. However, variations in preservation and diagnostic consistency must be considered.
These initial findings suggest a potential epidemiological transition during the Romanization of central Italy. Ongoing analysis, including forthcoming data from Sulmona and Alfedena, will provide a more robust framework for interpreting these health trends in their historical and socio-economic context.

The Sangro Valley: An Integrated Project for Anthropological and Cultural Enhancement

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The Sangro Valley, located between Abruzzo and Molise, is a territory of extraordinary landscape and cultural diversity. Thanks to its strategic position and the natural and human resources available, it has been, since ancient times, a crossroads of commercial exchanges and a site of significant political and economic interest. The numerous archaeological findings, including necropolises and individual burials, testify to the importance of this area but leave many questions unanswered about the communities that inhabited it.

Although archaeological documentation is well developed, there is a lack of in-depth anthropological studies capable of reconstructing the social and cultural dynamics of the Valley. This doctoral project aims to fill this gap, starting with a systematic survey of the available anthropological materials. Through an interdisciplinary approach, the project seeks to extract new insights into the human groups that lived and interacted within this territory.

At the same time, the project seeks to promote and enhance the Valley's resources through an integrated network of physical and virtual routes. Collaborations already established with transport companies and local institutions will enable the development of itineraries designed to make archaeological and anthropological sites accessible to a diverse audience, fostering an engaging and personalized experience.

The ultimate goal is twofold: on the one hand, to advance the scientific understanding of the territory; on the other, to overcome the area's tourism and infrastructural challenges by creating a cultural circuit that strengthens the Sangro Valley's economic and social potential.

"Di là dal fiume e tra gli alberi": Tomb 1 of Guardamiglio (Lodi, Lombardy) and its Topographical and Anthropological Context

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The Lower Lodigiano region, delimited by the Lambro, Po, and Adda rivers, presents a landscape shaped by fluvial action.

The Guardamiglio area is situated within the Po's meandering zone. Despite the presence of toponyms suggesting crossings and settlements linked to the hydrographic environment, archaeological evidence is scarce and primarily associated with preventative and emergency archaeology interventions. Indeed, the objective of this study is to present the results of the archaeological assistance conducted during the works for a gas pipeline, which brought to light funerary evidence, designated as Tomb 1.

The archaeological investigation, in the locality of Foppa, was conducted under the scientific supervision of the "Soprintendenza Archeologica, Belle Arti e Paesaggio per le province di Cremona, Mantova e Lodi". Through continuous monitoring along the gas pipeline's route, the average depth of the excavations reached -2.00 m below the modern ground surface, with deeper probes in specific locations. The discovery of anthropogenic evidence, concentrated in a single area, necessitated the opening of two trenches for the stratigraphic excavation of the inhumation and the documentation of adjacent features and finds.

Tomb 1 is an inhumation in a simple pit, oriented NW-SE. The individual is deposited supine with hands placed on the pubis. In the vicinity of the burial, other anthropogenic traces were identified, including cuts in the ground with carbonaceous fills, a possible posthole, and a feature interpretable as a ditch or channel.

The discovery of Tomb 1 and the associated features suggests a frequentation of the territory, possibly aimed at the seasonal exploitation of resources during periods of fluvial low water. In these periods, it is plausible to hypothesize a co-presence of spaces designated for craft activities and funerary use, generally assignable to the period between Late Antiquity and the Early Middle Ages.

Analysis of the recovered materials, in collaboration with the facilities provided by the Comune di Somaglia, particularly the steatite (pietra ollare), and the definition of the biological profile of the buried individual will provide elements for a better understanding of the populations who inhabited this territory and their interactions with a dynamic fluvial environment, integrating archaeological and anthropological data with historical and toponymastic information.

Nuraghe Su Angiu: a bioarchaeological perspective

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The reuse of earlier monumental structures is a widespread phenomenon in archaeology, reflecting diverse cultural practices across time. The motivations for such reuse remain uncertain, ranging from pragmatic to culturally symbolic. While cultural memory and the reappropriation of ancestral landscapes may often play a role, reuse frequently stemmed from practical and opportunistic motivations. Existing structures offered readily available, durable architecture, typically located in strategically or topographically favorable positions. Their material and spatial features made them suitable for various secondary uses – whether funerary, ritual, living, or military – resulting in complex palimpsests of activity.

In Sardinia, Nuragic architecture was often repeatedly reutilized from protohistory into the historical period. This study examines the Bronze Age quadrilobate Nuraghe "Su Angiu," near Mandas (SU), which was incorporated into a settlement that remained active at least until the fourth or fifth century AD. The reuse analyzed here is an episode of funerary use that pertains to the Sardinian Byzantine Era, which spans the 6th to 9th centuries AD. Excavations conducted from 2023 to the present have led to the recovery of commingled remains, archaeologically dated to the 8th century AD.

The evaluation of the osteological remains conducted thus far has determined that they belong to a minimum of ten individuals, who were apparently primarily buried in the Nuragic structure and heavily disturbed by later human activities. The assemblage includes both sexes, age spanning from young adult to mature. Macroscopic and microscopic analyses have evidenced the presence of healed cranial trauma, rare morphological variants (cervical ribs), and erosive and proliferative bony reactions compatible with systemic inflammation, possibly tuberculosis or brucellosis. A detailed differential diagnosis and systematic evaluation of these results will aid in reconstructing the osteobiographies and health status of the buried individuals.

Through detailed taphonomic and thanatological analyses, this study aims at contributing to our knowledge of Byzantine mortuary practices in Sardinia, focusing – through a bioarchaeological perspective – on how communities engaged with prominent monuments in the landscape – how these were perceived, repurposed, and assigned new functions – within a broader context of social, political, and economic instability and reorganization on the island.

Evaluating methodological consistency between metric and non-metric dental traits: a comparative approach

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In anthropology, the study of metric and non-metric traits is a key element in understanding biological relationships among ancient populations, their migration dynamics, human evolution, and genetic variability. Although qualitative and quantitative morphological traits are commonly analysed separately, owing to their differing nature and the distinct statistical approaches required, assessing their methodological congruence is critical to quantifying the biological distance between the populations analysed.

In this study, we analysed the coherence between metric and non-metric dental data from the Terramare necropolises in the Verona area, dating from the Bronze Age (c. 1500–1000 BC). Metric features were analysed using the Mahalanobis distance, which takes into account inter-variable covariance, while non-metric features, coded as binary presence/absence data, were analysed using the Gower coefficient, a method suitable for comparing different types of data.

We tested several analytical scenarios: (1) individuals grouped by site, and (2) individuals treated as separate units regardless of group affiliation. Although the strength and significance of the correlation varied between the scenarios, the overall results suggest a significant degree of convergence between the two types of data. Non-Metric Multidimensional Scaling (NMDS) visualisations further supported this pattern by revealing comparable clustering trends across datasets.

These results support the value of integrating metric and non-metric traits in bioarchaeological research, demonstrating that, under appropriate conditions, both can reflect comparable signals of population structures, despite differences in data type and measurement scale. This convergence highlights the potential of integrated approaches for reconstructing ancient biological relationships, especially in cases where only one type of morphological data is available. The combined use of Mahalanobis and Gower distances, complemented by the Mantel test, provides a robust and flexible framework for assessing methodological reliability in biodistance studies.

Paleoradiological and paleopathological investigations of Paolo Gorini's embalmed non-adult mummies (19th Century CE, Lodi, Lombardy, Italy)

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The scientific investigation of mummified and embalmed human remains has long been a cornerstone of bioarchaeological and paleopathological research, with significant methodological advances in recent decades. Within this field, the study of non-adult individuals is particularly important, as children's remains serve as critical indicators of population health, environmental factors, and maternal well-being. However, embalmed child mummies are rare, limiting our understanding of their preservation and diagnostic potential. This research focuses on five non-adult mummies embalmed using the petrification method pioneered by Paolo Gorini in 19th-century Italy. These individuals were analyzed using conventional radiography for the first time, enabling a comprehensive evaluation of the strengths and constraints of this technique when applied to petrified specimens. In parallel, both macroscopic and microscopic assessments were conducted on preserved skin to assess possible insect activity and postmortem changes. The results provide new perspectives on Gorini's embalming technique, particularly its implementation on juvenile bodies—a subject previously known primarily through physical specimens and scant archival notes. Radiographic evidence further revealed how Gorini may have adapted his methods in response to difficult cases. In addition, the skeletal analysis of these individuals offered crucial paleobiological data, including signs of developmental disruption probably consistent with vitamin D deficiency, shedding light on both embalming practice and the health profiles of these young subjects.

Bioarchaeological investigation of the remains of Blessed Bartolomeo Aiutamicristo (†1224): insights from a Canonical Reconnaissance

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The canonical reconnaissance of saints and blesseds of the Catholic Church offers a rare opportunity to examine ancient human remains, enabling multidisciplinary bioarchaeological research. This study focuses on the remains of Blessed Bartolomeo Aiutamicristo (†1224), a Camaldolese monk from a noble Pisan family, whose body has been preserved in the Church of San Frediano in Pisa. Initially kept beneath an altar, his remains were partially damaged in a fire in 1675, then reassembled and returned to public veneration between the late 18th and early 19th centuries.

In 2025, a canonical reconnaissance enabled the reconstruction of the biological profile of the individual through a multidisciplinary approach integrating macroscopic analysis, radiological imaging, stable isotope analysis, and dental calculus examination. Sex estimation based on cranial and pelvic morphology confirmed the individual as male, aged over 50 years at death, with a stature of 157 cm. The skeleton displayed robust muscle attachment sites, indicative of an active lifestyle. Paleopathological evidence included extensive dental disease, bilateral shoulder osteoarthritis, and vertebral degeneration.

Analysis of dental calculus revealed fragments of animal tissue associated with diet (possibly fish or meat), along with a variety of plant-derived elements that may be linked not only to dietary habits but also to living conditions. Stable isotope data confirmed a protein-rich diet derived from various sources, including meat, fish, and plants.

The body was found to be entirely skeletonized, with the bones reassembled in anatomical position. Notably, wooden replicas of several bones—including the right humerus and tibia, left patella and fibula, and multiple phalanges—replaced elements likely destroyed in the 1675 fire, as suggested by blackened skeletal fragments. These anatomically accurate replicas, along with the precise arrangement of the skeleton using wires and gauze, indicate the involvement of anatomists, possibly from the circle of Giovanni Paolo Mascagni (1755–1815), professor of Anatomy in Pisa.

This case study demonstrates the anthropological value of canonical recognitions, which, although rooted in religious tradition, offer unique opportunities for the investigation of individuals who occupied significant historical and cultural roles, contributing not only to the study of past human biology, but also to the interpretation of ritual, identity, and memory in Christian Europe.

Case of medieval cranial trephination in Siena

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At the end of March 2023, during the construction and operation of a low voltage power line along the itinerary of the Via Francigena, two tombs were brought to light at the church of SS. Vincenzo and Anastasio, in the quarter of Camollia, in Siena. From a historical point of view and in relation to the development of the pieve, the tombs can be dated back between 1087 and 1141 AD. The subject of this paper is the individual found inside the tomb 1. Sex determination was assessed by analysing the dimorphic traits of skull and pelvis; age at death was estimated through the degenerative changes on the articular surfaces of the first ribs and pelvis. Stature and body mass of the individual were calculated from the maximum length of the diaphysis and the head diameter of both femurs, respectively. For this reason, the individual of tomb 1is probably referred as and adult male with an age at death ranging between 35-50 years old, with a best point of 43 years. The analysis of body proportion yielded a stature of approximately 170 cm and a body mass of 68 kg, while the analysis of enthesis revealed a particular muscular strength in the distal elements of upper and lower limbs. The presence of specifical osteological traits found on the femurs and pelvis would suggest that the individual, during his lifetime, regularly practised horse-riding. A careful analysis of the cranial morphology has highlighted a regular bone opening of 2 cm x 2 cm located on the left fronto-parietal position, probably due to a trephination. The drill injury was analysed by computed tomography scan (CT scan). Inside and slightly below the hole there is a bony disc that was probably re-implanted; the bone remodelling surrounding the lesion indicates that the individual would have survived some time after the operation.

The Mystery of the Haifa Tomb: Analysis of the inhumates recovered from a hidden crypt of the San Paolo Church, Ferrara.

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In July 2023, a frescoed crypt containing numerous human skeletal remains was discovered during the renovation of the 16 th -18 th century Church of San Paolo in Ferrara, which provided an unexpected insight into early modern funerary customs. This study aims to investigate the biological and cultural features of the human remains found in the crypt, combining the archaeological context with anthropological and archaeo-entomological analyses.

The research involved the recovery and examination of human skeletal remains found in primary and secondary deposition. A combination of morphological and metric methods was employed to estimate sex, age at death, and stature. The materials were also examined for the presence of pathological lesion and taphonomic changes. An archaeo-entomological analysis was conducted to identify insect species associated with the stages of decomposition, which provided additional data on burial conditions and seasonal dynamics.

The skeletal assemblage is characterized by significant fragmentation and variable preservation, with evidence of both adult and subadult individuals.

The data obtained through this multidisciplinary analysis suggest a primary deposition with repeated use and reopening of the crypt over time, indicating a complex and dynamic use of space.

This interdisciplinary approach seeks to address issues of identity, social status and ritual in post-medieval Ferrara. The crypt is thus shown to be not only a burial space, but also a narrative fragment of a forgotten social and cultural landscape.

Functional Stress and Adaptations of the Cervical Vertebrae in Ancient Samples from Sicily: A Study on the Odontoid Process

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This study investigates the morphology and variability of the odontoid process of the second cervical vertebra (axis), a crucial element for the biomechanics of the atlantoaxial joint, in two ancient osteological samples from the Sicilian necropolises of "Ponte della Paolina" (Early Bronze Age) and Baucina (Archaic Age).

The primary objective is to document intra- and inter-population morphological variability and to evaluate possible correlations with functional stress or biomechanical adaptations related to occupational activities.

Methodology integrated osteological techniques, three-dimensional imaging, and radiological analysis. Thirty C2 vertebrae analyzed using 3D scanners, digital X-rays (clinical dentistry), supported by software for statistical analysis. Measurements: height, diameter, and width of the odontoid process; dimensions of vertebral body and articular facets. Morphological analysis identified variants (dens rectilineus, retroflexed, "crown-shaped"), and evaluated osteophytic alterations. Preliminary results indicate greater variability in odontoid process measurements in the Ponte della Paolina population compared to Baucina, with slightly higher average values in some dimensions. A higher incidence of osteophytic alterations was observed in the Ponte della Paolina remains, and a prevalence of retroflexed configurations in those from Baucina.

These morphometric differences and the observed association between the "crown-shaped" morphology and signs attributable to chronic cervical functional stress suggest potential mechanical adaptations to specific occupational loads. It is hypothesized that activities such as carrying weights or grinding may have influenced dens morphology. The morphometric variations of the dens appear not merely as anomalies but as potential reflections of adaptive strategies, functional stress, and possible hereditary traits.

This study highlights the effectiveness of an integrated multidisciplinary approach for the functional and evolutionary interpretation of the cranio-cervical region in archaeological contexts. The incorporation of advanced digital and forensic dentistry devices, such as oral scanners and dental X-ray machines, has proven invaluable, offering precise measurements and detailed imaging that enhance our understanding of historical human adaptations. This opens new perspectives for future insights into the influence of life practices on skeletal morphology.

Age-related changes in maxillary shape and bone remodelling throughout adulthood

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Age-related changes in adult facial morphology are marked by progressive reduced facial projection, increased facial concavity, and alterations in craniofacial proportions, reflecting key aspects of craniofacial senescence. These changes are due to bone remodelling, the continuous process by which old bone tissue is resorbed and replaced. While extensive jawbone resorption in older adults is often linked to tooth loss, the broader influence of aging on facial bone remodelling in adulthood remains poorly understood. This contribution explores the relationship between age, morphology, and bone remodelling in an adult sample of known age and sex from the Museum of Anthropology and Ethology of the University of Florence, Italy. We focus on the maxillary region using an integrative approach that combines microscopic and macroscopic analytical methods.

The sample, equally distributed between males and females was divided into four age groups: 20-35 (AG1), 36-50 (AG2), 51-73 (AG3), and 74+ years (AG4). High resolution casts of maxillary bone surfaces were produce using epoxy resin and analysed under a digital optical microscope. Surface histology was employed to visualize and quantify remodelling activities on the maxilla ($N = 24$). Mean bone remodelling maps were generated for each age group and compared with their respective mean shapes derived from a geometric morphometric analysis ($N = 39$).

On the microscopic scale, our results show that bone resorption was highest in the youngest adults (mean AG1= 25,77%), declined in intermediate age classes (mean AG2 = 12,15%; mean AG3= 11,75%), but appears to increase again in older individuals across both sexes (mean AG4: 30,57%). Results of the shapes analysis highlight that maxillary bone morphology is largely preserved in the younger age groups (AG1 And 2). In contrast, individuals in AG3 and 4 show a decrease in height in the maxillary arcade, likely associated with the higher incidence of antemortem tooth loss observed in these groups.

Our findings suggest that midfacial aging reflects the combined effects of localized remodelling due to tooth loss and systemic age-related increases in bone resorption. Notably, resorption continues to occur in regions consistent with those active in subadults, indicating a conserved spatial pattern of bone remodelling throughout life.

Testing commercial AI tools for Palaeopathology: potential and limitations of a case-assessed approach

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The rapid advancement of conversational artificial intelligence (AI) is transforming the field of medicine, particularly the way diagnoses and treatments are performed. Conversational AI models, especially pre-trained generative transformers such as the commercially available ChatGPT, are based on deep learning architectures trained on extensive datasets that include articles, websites, and various textual sources. In medicine, such AI systems have demonstrated potential as auxiliary tools for diagnosing typical and mildly atypical manifestations of common diseases. In the field of palaeopathology, differential diagnosis (DD) is a problem-solving process that sistematically evaluates potential diagnostic options while eliminating less plausible conditions. Given the inherently imperfect correlation between skeletal manifestations and specific diseases, palaeopathological diagnosis remains a complex undertaking. In this study, we explored the ability of commercial AI systems to assist DD. This investigation focused on case studies involving two young individuals, (both between 11 and 14 years of age), from a archaeological contexts dating to the Roman Imperial period and the Late Middle Ages who exhibited several lesions on both the cranial and postcranial skeleton. An experienced anthropologist conducted a rigorous DD, employing methodologies adapted from clinical medicine for the analysis of the archaeological remains. Subsequently, three commercially available and user-accessible AI chatbots, GEMINI (Google), ChatGPT (OpenAI), and Copilot (Microsoft), were evaluated. These systems were presented with a series of Information Sets. The AI systems were tasked with identifying known disorders across various body systems that could account for, or contribute to, the observed anomalies. They were also asked to propose a plausible DD. Then the diagnostic results were progressively refined by iterative querying, aiming to reduce the list to as few well-supported diagnoses as possible. The findings of this study revealed minimal differences among AI models in terms of scientific rigour and ability to generate plausible DDs comparable to those produced by the expert anthropologist. These outcomes are noteworthy and offer valuable insight into the potential role of AI in supporting decision-making processes in palaeopathological diagnostics.

Bioarchaeology of Childhood at Nora: Histological and Histomorphometric Analyses to Understand Growth Patterns in the Phoenician-Punic Necropolis

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The study of infancy in bioarchaeology is essential for understanding the evolution of life history in ancient human societies. Infant growth, health and mortality provide critical insights into population dynamics, cultural practices and the adaptive capacity of past communities. Among human remains from archaeological contexts, teeth are particularly informative due to their incremental growth microstructures, which preserve detailed chronological records of physiological stress, dietary changes, mobility and developmental patterns. Among the possible analytical approaches, dental histomorphometry coupled with biogeochemical analysis of dental enamel allows the reconstruction of an individual's biological life history. This study presents the preliminary results of dental histological analysis of 13 inhumed individuals (6 subadults and 7 adults) from the Phoenician and Punic necropolis of Nora (Cagliari, 7th – 4th centuries BCE). In recent years, excavations carried out by the University of Padova uncovered numerous burial contexts, revealing the necropolis to be a complex, stratified palimpsest containing both inhumations and cremations and reflecting cultural transitions from Phoenician to Punic influence. The sample includes both deciduous and permanent teeth, allowing a comparative approach: subadults, represented by their deciduous teeth, provide a snapshot of those who did not survive childhood, while early life of those who survived to the adult stage is studied through their first permanent molars.

A histomorphometric analysis of dental enamel was conducted to determine the crown formation times, the rates of enamel daily secretion and extension, and the presence of physiological stresses manifested as Accentuated Lines.

This dual framework allows us to explore the relationship between early life stress and later health implications. Furthermore, from an archaeological perspective, it also allows diachronic comparisons of health and growth conditions across the Phoenician and Punic phases.

Our findings enhance the understanding of growth patterns, physiological stress, and health conditions among infants in Phoenician and Punic societies. When compared to coeval sites, such as Motya (7th–6th centuries BCE, Sicily), they contribute to a broader interpretation concerning demographic dynamics and adaptive strategies of these communities across the western Mediterranean.

Intentional traumatic injuries related to three individuals from the cemeterial area of the Castle of Miranduolo (XI-XII century)

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The burials analysed belong to the population of the Castle of Miranduolo in the period between the end of the 11th and the beginning of the 12th century. In these decades, before 1133, Miranduolo was besieged by the bishop of Volterra, engaged in a biting conflict against the Gherardeschi counts for control of the Val di Merse. The cemetery stood next to the church in perfectly squared ashlars, paved in stone pieces and provided with a presbytery enclosure. On its exterior, two walls delimited the cemetery space; here an active and stratified community is revealed, divided between members of the élite, men-at-arms, and peasant families. Osteological analysis of the human remains from the cemetery area have highlighted significant traumatic cranial injuries on three adult male individuals (SK 69, SK 79, SK 86). The present work, supported by tomographic investigations, analyses and identifies these injuries as the result of intentional wounds caused by blunt and sharp weapons.

Reconstructing mobility in Bronze Age North-East Italian Communities: Strontium Isotope (Sr/Sr) Analysis of Dental Enamel

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Within the PRIN 2022 SHOVEL framework, this study investigates mobility and provenance among Bronze Age Terramare populations in Northern Italy. By measuring Sr/Sr ratios in dental enamel against local environmental baselines, we assess patterns of residence, regional exchange, and community connectivity.

Given the unusually high incidence of shovel-shaped incisors in these communities, we examined whether individuals possessing this trait exhibit distinct movement histories compared to those without it.

Enamel from 55 human individuals and 14 faunal specimens was collected across five necropoleis—Olmo di Nogara, Bovolone, Ballabio, Franzine Nuove, and Castello del Tartaro.

Soil and animal remains defined the local bioavailable strontium baseline, enabling us to distinguish local from non-local signatures. The results show that most individuals fall within the local isotopic range, indicating residential continuity.

A minority exhibits divergent values, reflecting episodes of movement between different groups. Comparative analysis reveals the overlap in isotope distributions between those with and without shovel-shaped incisors, suggesting no direct link between this dental trait and childhood provenance.

To better understand geographic origins, human data were modeled against regional bioavailable strontium isoscapes using probabilistic assignment frameworks. Isotopic provenance was then correlated with demographic factors, funerary treatment, and grave goods to explore possible social integration and residence rules.

These results contribute to debates on territoriality, exchange networks, and community organization in Bronze Age Po Valley societies. Under the SHOVEL project, enamel-based strontium isotope analysis, integrated with other isotopic and molecular analyses, underlines the power of bioarchaeology to reconstruct individual life histories and social dynamics in prehistoric Northern Italy.

From the individual to the community of the dead: taphonomic complexity at the western necropolis of Nora

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Funerary rituals constitute one of the most emblematic expressions of the cultural, social, and symbolic dynamics within a community. Each decision concerning the treatment of the deceased - ranging from the typology of burial structures, the number of individuals interred, to the positioning and orientation of the bodies - responds to well-defined logics that reflect social structures, religious beliefs, and culturally embedded practices. In this sense, the spatial and ritual organization of a necropolis offers a privileged framework for reading and interpreting both collective and individual identities of past societies.

Within this perspective, the western necropolis of Nora (Sardinia, CA), currently investigated by the University of Padua, stands out as a key case study in the central-western Mediterranean for investigating funerary practices during the late Phoenician and the Punic periods. The site shows evidence of long-term use and considerable ritual complexity, making it a valuable context for examining burial customs in a region marked by intense cultural interactions.

The excavation of 11 inhumation tombs, archaeologically dated between the late 7th and the 3rd centuries BCE, has revealed nuanced evidence. Through a multidisciplinary approach - integrating archaeological, anthropological, and taphonomic data - the research has documented substantial variability in the use of funerary space, depositional sequences, and the treatment of the dead. Marked differences were identified in both the architectural configuration and reuse of burial structures, as well as in the funerary gesture, including body positioning, orientation, and the treatment of human remains over time.

The coexistence of distinct funerary practices within the same necropolis - and at times within the same tomb - reflects the presence of a complex and dynamic community. This heterogeneity suggests a flexible use of funerary space and of ritual protocols, likely shaped by factors such as kinship, age, sex, and status.

Unpublished Anthropological and Paleopathological Analyses of the Population of the Roman Port of San Gaetano (Vada Volaterrana, LI) During the Late Antiquity–Early Middle Ages

Giulia Saviano, Paolo Sangriso , Silvia Marini , Simonetta Menchelli

In the 1st century AD, the site of San Gaetano was built as part of the port system of Vada Volaterrana, which belonged to the city of Volterra. Between Late Antiquity and the Early Middle Ages, the district was abandoned and transformed into a burial area. To date, 32 burials have been uncovered, and their analysis aimed to reconstruct the living conditions, health, and socio-labor organization of this coastal community. The sample, consisting of 18 adults and 14 subadults, was studied using established methods for determining sex and age, as well as for identifying dental pathologies, physiological stress markers, enthesopathies, and signs of muscular activity.

Adults were buried in simple pits or cappuccina-style graves, while subadults were buried in amphorae, revealing funerary rituals that granted even newborns social recognition.

The sample shows a slight predominance of women and a lack of individuals aged 20 to 29, along with adolescent mortality occurring exclusively among females—possibly indicating risks associated with pregnancy or childbirth.

Widespread dental pathologies reflect a diet rich in carbohydrates and poor oral hygiene. The presence of cribra orbitalia, enamel hypoplasia, and periosteal reactions indicate nutritional deficiencies, infections, and poor hygienic conditions, with particular vulnerability noted between ages 3 and 4, during the post-weaning phase.

Musculoskeletal analysis reveals a clear gender-based division of labor. Women display markers associated with repetitive upper limb movements and kneeling postures, compatible with domestic or agricultural tasks; men exhibit skeletal changes linked to stress on the lower limbs and prolonged walking.

Both sexes show signs of vertebral osteoarthritis, Schmorl's nodes, and other overload-related pathologies. Additional skeletal alterations were identified, including an osteoma, suspected idiopathic skeletal hyperostosis, and endocranial lesions potentially attributable to tuberculous meningitis.

Despite the fragmentary nature of the remains, the data provide valuable insights into the lifestyle of a population from an early medieval coastal context in Tyrrhenian Italy, marked by harsh environmental conditions, nutritional imbalances, and a well-defined social structure that also considered the youngest members of the community.

The burial in the baths: an uncertain deposition in the roman “Domus dei mosaici marini”, Porto Torres (SS)

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Turris Libisonis (modern-day Porto Torres) was the first Roman city established in Sardinia, founded in 1st century BCE by the Gens Julia. Located on the city’s western side were the Pallotino thermal baths and several private buildings, such as the so-called Domus dei Mosaici Marini (Marine Mosaics), named for the numerous fish depictions inside.
This structure, excavated by the archaeological superintendence for Sassari and Nuoro, underwent multiple phases of occupation and functional transformation between the 3rd and 5th centuries CE.
On the western side of the building, a distinct space had three phases of use:
1.Thermal/ Residential Phase (3rd century CE)
2.Paleo-Christian Phase (4th century CE)
3.Industrial Phase (5th century CE)
Tomb 2 was located on the western side of this final rectangular space, directly above the Paleo-Christian floor. The stratigraphic context suggests that the burial occurred during the transitional period between the abandonment of religious functions and the establishment of industrial activities.
The purpose of this study is to understand who this individual is and why the reason for his burial in this area.
The burial was an inhumation in an earth-cut grave with well-defined edges. It contained a single individual interred in a primary right-sided lateral position, oriented along a southwest-northeast axis. This specific positioning appears to have been more frequently associated with non-local individuals during this period.
The osteological analysis identified the individual as an adult male, with an estimated age at death between 35 and 45 years. Stature estimation suggests a height of approximately 175 cm. Pathological assessments revealed the presence of small, poorly defined destructive lesions (¡2 cm in diameter) with some reactive sclerosis on the internal cranial vault of the parietal bones. Additionally, an osteoma was observed on the external right parietal bone.
The left petrous portion of the temporal bone was sampled and submitted to the Archaeo and Palaeogenetics group at Institute for Archaeological Sciences of the University of Tübingen (Germany) for ancient DNA analysis. Shotgun screening results reveal that the ancient DNA in this specimen is well preserved, with 18.9% of human DNA and around 35% of CtoT substitutions at the molecule termini. Further genome-wide analyses are currently in progress.
A multiple inhumation, belonging to third phase, was identified on the eastern side of the same room.

A new online database of virtual cranial remains from Sardinia, from prehistory to modern times

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We are pleased to introduce *The Collection of Sardinian Crania from the Museo Sardo di Antropologia ed Etnografia (MSAE), University of Cagliari*, a newly established open-access digital repository featuring high-resolution 3D models of human crania from prehistoric and historic periods of Sardinia. This curated collection, hosted on the online platform MorphoSource, includes 416 cranial specimens spanning from the Neolithic to the 20th century. With increasing reliance on virtual osteological collections for interdisciplinary studies, this project contributes substantially to advancing data-driven research in human skeletal biology.

The collection derives primarily from mid-20th-century excavation campaigns by the museum's founder and collaborators. These efforts recovered human remains from multiple archaeological sites across Sardinia. The crania, now curated at the MSAE, represents diverse chronological and geographic contexts within the island, offering a rare opportunity to explore long-term biological variation and population history in a Mediterranean setting. Digitalization and public dissemination were made possible through authorization from Sardinian cultural heritage authorities.

The 3D models were created using ultra close-range digital photogrammetry (UCR-DP), a non-invasive imaging method enabling accurate capture of external morphological features. Photographs were taken with a DSLR camera setup and processed via cloud-based photogrammetric software. Each model was scaled using repeated measurements between anatomical landmarks, allowing precise morphometric analysis. This process was conducted by undergraduate and doctoral students as part of their thesis work, integrating training and data production within an academic framework.

In addition to preserving and democratizing access to this important collection, the database is already supporting new scientific work. We anticipate this resource will stimulate a wide range of future investigations, including studies of cranial morphology, pathology, population dynamics, and heritage conservation. We encourage the international research community to engage with this collection as a tool for collaborative exploration of Sardinia's complex human history. By offering a standardized, scalable dataset, this initiative supports reproducible research and promotes broader engagement with Mediterranean bioarchaeological heritage.

Funerary rituals in the Recent Bronze Age of the Veneto region: a bioarchaeological study of the necropolis of Franzine Nuove di Villa Bartolomea (VR), 1350-1000 BCE

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Funerary archaeology represents an essential tool for the historical reconstruction of past societies, since the analysis of burial practices, including the distribution of tombs, the study of human remains and the examination of grave goods, provides valuable information on the individuals, which includes age at death, sex, social status, wealth and beliefs. This study focuses on the biritual necropolis of Franzine Nuove di Villa Bartolomea (VR), one of the most important Bronze Age sites in Northern Italy, dated between 1350 and 1000 BCE. The site was discovered in 1968 during excavation work for the construction of a corn drying plant, and archaeological research was carried out from 1968 to 1983 by the Civic Museum of Natural History of Verona, in collaboration with the Archaeological Superintendence of Veneto, bringing to light 582 burials arranged on two levels of the ground. The aim of this study was to investigate possible correlations between the biological profile of the individuals and the burial ritual, with particular attention to the position of the body at the time of deposition. In this study, 117 individuals were analysed. For each skeleton, the biological profile (sex and age estimation) was determined, paleopathological analysis was carried out on bones and teeth, osteometric indices were calculated, and statistical analyses were conducted to assess the distribution of biological profiles in relation to funerary rituals. Furthermore, the presence of non-metric traits on the skull and teeth was also observed to evaluate particular aspects related to the different types of burial found. The sample consisted of 54 subadults and 60 adults, including 18 males and 33 females. Sex could not be determined for 9 adults or any of the subadults. In 3 cases, both sex and age could not be assessed due to missing key skeletal elements and severe fragmentation of the remains. From a paleopathological perspective, several cases of metabolic and biomechanical stress emerged. The comparison of the biological profile and burial position highlighted the adoption of different funerary rituals according to the sex and age of the individuals, aligning with the evidence brought to light in other contemporary Veronese necropolises. This study contributes to the understanding of social and funerary dynamics within Bronze Age communities of Northern Italy, highlighting the importance of integrating osteoarchaeological data with ritual context.

Trophy Skulls from Papua: Interdisciplinary Insights into Decoration, Preservation, and Cultural Meaning

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The practice of preserving skulls as trophies, relics, or ritual objects is a widespread phenomenon documented across various cultures and geographic regions. In Indonesia and New Guinea, indigenous population used to preserve crania of fallen enemies after cleaning and decorating them with pigment and incisions. Such prepared skulls are commonly referred to as "trophy skulls."

Approximately 80 such specimens are held in the Anthropology and Ethnology collection of the Museum of Natural History in Florence, mostly collected by Lamberto Loria between 1911 and 1914, and primarily originating from the D'Entrecasteaux Islands and the Gulf of Papua. These skulls typically display different type of incisions on the frontal bone, with the mandible secured to the cranium using various intricate bindings made of vegetal fibers. Several specimens also show traces of pigments, likely ochre. In some cases, teeth were replaced with wooden substitutes, while in rare instances—particularly in skulls with complete or nearly complete dentitions—cordage was used to hold the teeth in place.

A detailed analysis of these trophy skulls was conducted through an interdisciplinary approach that integrates anthropological, conservation, and ethnographic perspectives, with the aim of: (i) reconstructing and evaluating potential correlations between incision, pigmentation, and cordage patterns and the biological characteristics, geographical origin, and cultural context of the individuals; and (ii) assessing material degradation and preservation conditions needs to support future conservation efforts.

The skulls were digitalized using Artec 3D scanner, and a dedicated cataloguing form was developed to record detailed information on the collectors, biological profile, pathologies and traumas, geographical origin, types of decorations, presence/absence of pigments, additional decorative elements (e.g., cordage and wooden teeth), as well as the overall preservation state.

Here we present preliminary results of this project. To date, 63 skulls have been digitized and studied: 32 identified as male, 29 as female, and 2 of undetermined sex. Age-at-death estimation indicates that 11 individuals were young adults, 41 were adults (probably up to 40 years old), 6 were mature adults, and 5 were children. Additionally, 26 skulls exhibited identical or highly similar incision patterns, potentially indicating shared cultural practices or a common symbolic meaning.

Tracing back a modern dental disease: a severe case of Molar Incisor Hypomineralisation (MIH) in an Imperial Roman Child (Isola Sacra, 1st-3rd century AD)

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Molar Incisor Hypomineralisation (MIH) is a pathological condition caused by a qualitative alteration in the mineralization process of dental enamel and currently occurs in about 13% of the world's population. Although its etiology is still unknown, it is believed that systemic prenatal diseases, vitamin deficiencies and metabolic imbalances could be involved. To date, a few studies have reported possible cases of MIH in ancient populations according to modern diagnostic criteria, but due to its complexity this anomaly remains largely undiagnosed, misdiagnosed or confused with taphonomic processes.

This study presents an ancient case of severe MIH identified in the in situ mandibular dental remains of an Imperial Roman child (SCR 6302) from the Isola Sacra necropolis (Portus Urbis Romae, Latium, 1st–3rd century AD), employing a comprehensive approach that integrates modern clinical classification methods, X-rays imaging (XRM) techniques and histomorphometric analysis.

MIH-induced lesions were detected in the second deciduous molars, manifested as a collapse of the occlusal surface with exposure of the dentine. XRM analysis revealed an early stage of pathology, evidenced by marked enamel hypoplasia in the first permanent molar and the presence of secondary caries on the first deciduous molar. Following histomorphometric analysis, the age at death was set at 2.5 years and the onset of MIH was circumscribed to the first months of life, thus highlighting the presence of non-specific physiological stresses in the pre- and postnatal enamel portion.

Through a multidisciplinary approach, the present study reports a confirmed case of severe MIH in an ancient population, revealing an earlier occurrence than typically reported in modern cases. Furthermore, it is suggested that the development of MIH may be driven by common or similar aetiological factors present in both past and contemporary populations, offering novel insights into the pathological processes underlying this condition.

Body size and sexual dimorphism of the coxal bone: allometric variation in two contemporary Italian populations

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Allometry investigates the biological scaling relationship between the size of a body part and the overall body size. This study aims to investigate whether sexual dimorphism of the coxal bone may be related to allometric differences in body size. If allometry is not responsible for the sexual dimorphism observed in a set of coxal bone metrical traits, we hypothesize that other factors — such as obstetric demands, environmental conditions, genetic background, hormonal influences, and biomechanical stability — may play a prominent role. A set of metric measurements of the coxal bone were recorded in a sample of 132 individuals from the two documented human osteological collections of Bologna and Sassari (Bologna = 64, Sassari = 68) housed at the University of Bologna. These two populations are coeval but originate from very different geographical regions (island vs. peninsula) and have distinct genetic backgrounds. Previous population studies report differences in stature, with Sardinians being generally shorter and North Italians being taller. Measurements of the maximum femoral length and the diameter of the femoral head were collected, and a geometric mean was calculated as a proxy of body size. Following log-transformation of the body size data and the pelvic measurements, a Standardized Major Axis (SMA) analysis was performed.

The results showed that a considerable number of dimorphic variables follow allometric patterns. Particularly, longitudinal measurements (such as M01: coxal bone maximum height) reflect scaling tendencies, indicating that individuals with larger body size tend to have proportionally bigger hip dimensions. Other variables display divergent trends between populations or the sexes. For instance, some measurements related to the width of the coxal bone (such as M04: coxal bone depth) scale with body size in males but not in females, suggesting an obstetrical constraint in females. Finally, differences in allometric patterns between Sassari and Bologna may be explained by the interplay of distinct environmental and genetic influences that characterised these two populations.

Sessione

Antropologia Molecolare

comunicazioni orali

Synthetic Genomic Data Augmentation for Underrepresented Populations Using Open-Source Large Language Models

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The lack of **high-quality genomic data** from underrepresented populations limits the accuracy and generalizability of population genetic inferences, such as those based on Identity by Descent (**IBDNe**). This study introduces a novel framework to augment Variant Call Format (**VCF**) datasets through the **fine-tuning** and deployment of an **open-source large language model** executed entirely on local computational infrastructure. Drawing on public repositories such as the 1000 Genomes Project, the method generates biologically realistic variant calls that enhance sample size and population diversity without compromising individual privacy or data ownership. The model is fine-tuned on high-confidence variant sites curated from established panels, allowing it to learn patterns of allele frequency, linkage disequilibrium, and haplotype structure. The resulting synthetic VCF entries are post-processed to ensure standard compliance and biological plausibility.

To ensure **privacy, copyright compliance**, and ethical integrity, all training and inference are performed exclusively on institutional servers. No genomic data are transmitted externally. Rare variants and individual genotypes with allele frequencies below a user-defined threshold are filtered out to prevent potential re-identification. Quantitative divergence metrics, including pairwise distance measures and low-dimensional embeddings, such as **PCA and UMAP**, are used to verify that synthetic genomes do not closely replicate any specific real sample. In accordance with best practices, only the trained model weights are shared, while the synthetic datasets remain confidential.

Applied to an underrepresented Eurasian population used as a test case, the augmentation process led to a marked increase in effective sample size while preserving key features such as principal component clustering and pairwise genetic distances. Hierarchical clustering and **IBDNe** analyses performed on the augmented dataset successfully reproduced the demographic signal of the original population within expected confidence intervals.

This resource-efficient and scalable approach improves the representation of diverse human groups in genomic research, enabling more robust analyses of population structure, demographic history, and disease associations. By integrating open-source language models with rigorous privacy safeguards, the proposed framework contributes to making genetic research more inclusive and trustworthy.

Human demography shapes patterns of language diversity across the world

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Human history is written in both our genomes and our languages. The extent to which our biological and linguistic histories align has been the subject of long-standing debate. Transmission with modification can shape genes and languages similarly, while horizontal contact can uniquely affect languages. Demographic contact typically leads to linguistic homogenization, but it can also lead to more or less deliberate processes of linguistic divergence. In turn, language boundaries can influence genetic structure acting as barriers to gene flow. While local case studies provide evidence to anchor specific cultural and demographic histories, we still lack a systematic, global perspective on how population histories and language diversification correspond, and how speakers' dynamics influence the diversity of languages and their features.

To address this, we use the GeLaTo (Genes and Languages Together) database, which links genome-wide data with linguistic identifiers for over 500 populations worldwide. We estimate patterns of genetic distance, excess of homozygosity and admixture to ask three key questions: (1) Do language families correspond to clusters of genetically related populations? (2) Are language isolates associated with genetic isolation? (3) How does demographic history—particularly admixture and isolation—shape structural features of languages? We find trends of genetic cohesion for speakers of the same language families (i.e. related languages that share a common ancestor), with around 20% of populations deviating from this pattern. We explore the contrast between expansive language families vs. language isolates, and find a tendency for genetically isolated populations to speak isolated languages. We finally examine the influence of genetic isolation and genetic contact (admixture) on diverse linguistic features using quantitative linguistic databases, and find that contact favors structural convergence, while genetic isolation correlates with structural diversification.

Our results support hypotheses in historical linguistics, sociolinguistics and linguistic typology, opening new avenues for studying processes of cultural evolution and cultural transmission across human populations.

Semi-super centenarians as a model to test the contribution of natural selection in modulating individuals' predisposition to longevity

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Human life expectancy has significantly increased in post-industrialized societies, largely due to advances in medicine, improved sanitation, better nutrition, and overall living conditions. These changes have particularly reduced the impact of infectious diseases on health and mortality, also pulling survival into extreme old age so that the prevalence of centenarians (i.e. 100 years old or more), semi-super centenarians (hereafter 105+, i.e. 105 years old or more) and super-centenarians (i.e. 110 years old or more) raised considerably. In particular, 105+ represents a relevant threshold, with individuals who reach such a milestone being characterized by an exceptional healthy phenotype. Since genetic factors contributing to human longevity can be considered environmental-specific and are supposed to have been influenced by the peculiar evolutionary history of each population, an evolutionary genomics approach promises to provide useful insights into the genetic determinants of this complex phenotype. Previous studies aimed at investigating the evolution of complex biological traits in human groups that contributed to the formation of the present-day Italian gene pool pointed to differential adaptive processes experienced by the main population clusters observable within the cline of variation distributed along the peninsula. Interestingly, some of the identified adaptive traits were proposed to have evolved in response to pathogen- and/or dietary-related selective pressures and have been mediated by combinations of variants at pleiotropic genes, which have the potential to influence also lifespan. Here we replicate these analyses on a cohort of 81 Italian semi-super centenarians and super-centenarians. In detail, we took advantage of information for around 15 million single nucleotide variants (SNVs) from high-coverage (90x) whole genome sequence data to detect gene networks and biological functions that might have been shaped by the action of natural selection. Then, we compared the identified signatures with those inferred from samples representative of the overall Italian population to test whether 105+ genomes are enriched for putative adaptive loci that may have secondarily modulated individuals' predisposition to delayed onset of age-related diseases, thus playing a key role in promoting extreme longevity.

Adaptive evolution of complex traits regulating insulin signalling and energy metabolism in brown and white adipose tissue mediated adaptation to extreme cold environment in Yakut populations from Northern Siberia

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Complex (i.e., polygenic) adaptive traits are supposed to be the bases of most human biological adaptations to environmental conditions, having allowed *H. sapiens* to occupy and stably live in a vast range of geographical areas including those characterized by harsh climate. Nevertheless, few methodologies have been developed so far to investigate the genetic determinants of these adaptive traits. Here, we coupled multiple methods with a machine learning-based approach to pinpoint combinations of genes simultaneously presenting i) weak signatures ascribable to the action of natural selection and ii) functions linked to the regulation of the same biological trait, as expected under a model of polygenic adaptation. Such a pipeline of analyses was applied to whole genome sequence data for individuals from Northern Siberia belonging to the Yakut ethnic group. The obtained results were compared with those for a population of related ancestry in order to focus on Yakut-specific adaptations and the potential involvement in adaptive introgression events of loci identified as targets of natural selection was tested by considering both Neanderthal and Denisovan sources of archaic alleles. Genes that play a role in the modulation of thyroid hormone signalling and glycerolipid metabolism, along with insulin-related loci, showed patterns of haplotype variation conform to a model of adaptive evolution. These genes are known to contribute to the regulation of insulin signalling and energy expenditure in white adipose tissue, as well as of the correct functioning of brown adipose tissue during cold exposure. In line with this evidence, a gene showing signatures of archaic adaptive introgression from Neanderthals was found to participate to lipids metabolic pathways, while the most validated signal supporting adaptive introgression from Denisovans was observed for a locus whose variants are associated to an increased risk of obesity, Type 2 Diabetes and insulin resistance. Overall, these biological functions were proved to be pervasively modulated during/after cold exposure in several species of mammals, suggesting that adaptive evolution of the underlying genes might have represented one the main driver of complex adaptations evolved by Yakut ancestors to cope with an extreme cold environment.

High-Resolution Kinship Inference with SNPs: A Forensic Tool for Complex and Distant Relationships

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Kinship recognition between anonymous DNA samples is becoming increasingly relevant in forensic science, particularly as national and international DNA databases continue to expand. While Short Tandem Repeats (STRs) remain the gold standard for close kinship analysis, their limitations in detecting distant relationships—due to high mutation rates and a restricted number of loci—necessitate more advanced approaches.

Next-generation sequencing (NGS) has emerged as a powerful tool for personal identification, enabling the simultaneous analysis of a wide range of genetic markers. In this context, we previously developed and evaluated a novel panel of 4,849 Single Nucleotide Polymorphisms (SNPs) specifically designed for high-resolution kinship inference on 150,000 simulated individual pairs, ranging from unrelated to fifth-degree relatives using a combination of the Forrel package in R and supervised machine learning algorithms.

The panel demonstrated strong performance in identifying kinships up to the third and fourth degrees, with recall values exceeding 0.6 even for fifth-degree relationships. Machine learning integration further enhanced accuracy, improving F1 scores by approximately 12.25% and 20% for fourth- and fifth-degree relationships, respectively. Notably, the method achieved over 99% accuracy in distinguishing related from unrelated individuals.

To assess real-world applicability, we evaluated the panel's performance using sequencing data from 2,386 individuals with documented kinship ties up to the third degree, available through the 1000 Genomes Project. Specifically, we processed 1,206 confirmed parent-child pairs, 15 sibling pairs, 36 grandparent-grandchild or avuncular pairs, and 29 third-degree relatives using a Python- and R-based pipelines.

Overall, this study highlights the potential of SNP-based panels, combined with advanced computational methods, to significantly improve kinship inference—especially in complex or distant relationships—offering a robust alternative to traditional STR-based approaches in modern forensic investigations. This approach is particularly valuable in mass disaster scenarios and missing person investigations, where establishing biological relationships is often the only viable path to identification.

Global distribution of the APOE haplotypes in different human populations

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The Apolipoprotein E (APOE) gene has emerged in multiple studies on human longevity and age-related diseases. APOE is expressed in cells of the liver, kidneys, adipose tissue, and immune and central nervous systems. The main role of the protein encoded by this gene is to facilitate the transport and uptake of lipoproteins and cholesterol.

The APOE protein exists in three isoforms (APOE2, APOE3, APOE4), which correspond to three different haplotypes (2, 3, and 4) defined by the combination of two polymorphisms (rs7412-C/T and rs429358-T/C).

These genetic variants reflect differences in amino acid sequence and these isoforms have markedly different functional properties and associations with human health. In particular, the 4 haplotype is negatively associated with longevity and linked to age-related cardiovascular and neurodegenerative diseases, such as Alzheimer's. Recent bio-anthropological studies showed that the 4 haplotype confer reproductive advantages to naturally fertile populations living in highly pathogenic environments. A north-south decreasing gradient of 4 frequency has been observed in Europe and Asia. Conversely, 2 is considered a pro-longevity allele with protective effects against Alzheimer's disease in certain human populations, although it is the least frequent worldwide. Finally, 3 is the most common and functionally neutral isoform.

Previous studies showed that the global variation in APOE allele frequencies reflects the combined effects of natural selection and demographic history. Although there are meta-analyses on the variability of this gene in the literature, it has not yet been fully characterized in many human populations.

This study aims to investigate the distribution of APOE haplotypes in different populations, to provide a full description of the biodiversity of these haplotypes with major implications in terms of health and longevity.

To this end, we selected DNA samples from cohorts of individuals from different human populations characterized by a high level of variations in terms of genetic ancestry, geography and ethnicity. We applied a TaqMan Real-Time PCR Assays to genotype the aforementioned SNPs. Together with data reported in the literature by several studies, the resulting data was used to create the most detailed map of natural variations on this haplotype to date, thus contributing to a broader understanding of its global variability.

Unveiling the Complex Genetic History of Italy from Paleolithic to Middle Age through Ancient Whole-Genome data

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The Italian Peninsula, a crossroads of Mediterranean cultures, has long been recognized for its pivotal role in European history. Despite extensive genomic studies across Europe, the genetic history of Italy remains largely underexplored due to scarcity of ancient whole-genome data spanning limited geographical regions. Here we present a comprehensive analysis of 60 shotgun-sequenced ancient genomes (average coverage of 2.12x) from 21 necropoleis across mainland Italy, Sicily, and Sardinia, spanning about 10,000 years of history, alongside hundreds previously published ancient complete genomes covering from the Paleolithic to the Medieval periods. Leveraging genotype likelihood computations and imputation of missing positions alongside the genome, we achieved robust statistical reconstructions of past evolutionary dynamics despite the inherent challenges of low-coverage data. Our results reveal a complex mosaic of external influences shaping the genetic landscape of Italy. After the arrival of the early farmers in Italy, we observed two different Neolithic-related ancestries highlighting a genetic structure possibly rooted in the Anatolian region, while the Bronze Age marks a significant genetic influx of Pontic Steppe related populations. This migration established a genetic gradient, with northern regions showing higher Steppe-related ancestries compared to the south of Italy. Additionally, we detect a significant genetic contribution of an Iranian Neolithic-related ancestry during the Iron Age, and particularly the Imperial period. These results shed light on the strategic position of the Italian Peninsula at the heart of the Mediterranean Sea, which allowed the arrival of different groups and cultures since prehistory. Phenotypic inference of pigmentation traits (eye, hair and skin color) reveals a shift toward lighter phenotypes beginning in the Iron Age. This study offers unprecedented insights into the genetic history of Italy, elucidating the interplay of migration, environment, and cultural evolution that shaped its rich heritage. These findings contribute to our understanding of the unique position of Italy in the broader context of European genetic history, highlighting the methodological innovations and interdisciplinary approaches necessary for exploring complex demographic dynamics.

Uncovering human mobility and genomic variability in Medieval Central Italy: Insights from ancient DNA and strontium isotope analyses

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During the Middle Ages, the Lazio region in Central Italy served as a strategic crossroad of different cultures. This study investigates two populations from this area: Santa Severa (7th–15th century CE), a key Mediterranean trade hub, and Allumiere-La Bianca (15th–16th century CE), among the earliest alum mining settlements in Italy.

To explore patterns of genetic diversity and individual mobility, we integrated ancient DNA (aDNA) analysis with strontium (Sr) isotope. Sr isotopes from tooth enamel reflect an individual's childhood geological environment, thus informing on geographical origins.

We extracted aDNA from teeth and petrous bones using a silica column-based protocol, and prepared double-stranded genomic libraries for shotgun sequencing. Low-coverage genomes (0.1X) were obtained for 69 individuals: 56 from Santa Severa and 13 from Allumiere-La Bianca. Moreover, from Santa Severa to assess genetic continuity over time we analysed 8 Etruscan (6th–3rd century BCE) and 5 Roman (3rd century BCE–5th century CE) individuals.

The Etruscan and Roman genomes from Santa Severa fall within the genetic variability of previously published Etruscans and Roman populations, distinct from the Italian Medieval samples (including individuals from Santa Severa). Moreover, between the Early Medieval population of Santa Severa and the Late Medieval population of Allumiere-La Bianca a genetic difference was also observed suggesting a geographical and temporal differentiation during the Middle Ages. Several Medieval individuals also exhibit non-local strontium isotope signatures, suggesting extra-regional origins.

Ongoing research will involve high-resolution analyses of ancestry and population structure through genotype imputation, including identity-by-descent (IBD) sharing, which is essential reconstructing the dynamics of recent populations.

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Genetic and Cultural Dynamics of post-Roman Central Europe

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The transition from Late antiquity to the Early Middle ages in Central Europe has traditionally been interpreted as a period of large scale migrations and conflicts between northern "barbarians" and the retreating fringes of the Roman Empire. To the very same timeframe, however, can be traced the origins of many modern towns and villages, as well as the appearance of new cultural practices.

In order to explore the cultural and demographic dynamics of this crucial phase, we generated 248 new whole genomes sequences from two regions in Southern Germany: the Danube-Isar area, which was part of the Roman Empire until the early 5th century, and the Rhine-Main area, that was abandoned at the end of the 3rd century. We focused on individuals from the 5th to the 7th century, but included Late Antiquity (3rd and 4th century) samples to provide insights into the Late Roman period.

Altheim-Essenbach, our main study site, was likely founded by a group with Northern European ancestry in the 5th century, and we detected a demographic shift in the following century triggered by the integration of newcomers with an ancestry that is typical of local roman[ised] settlements. By the 7th century, the fusion of these diverse ancestries had created a genetic diversity resembling the one still observed in modern Germany. We reconstructed a large pedigree network spanning up to seven generations and developed a novel strategy to infer the ancestry of unsampled individuals, which allowed us to identify immediate intermarriage between local and incoming groups.

Our results highlight rapid integration and cultural assimilation of groups with distinct ancestries following the downfall of roman power in the area; and show that these Early Medieval communities were organised around small families exhibiting a bilateral or loosely patrilineal descent, avoided kin marriages and practiced monogamy. These cultural practices, that began during the Late Roman period, will go on to influence Europe in the centuries to come.

Ancient and archaic mobile elements underline global evolutionary trends and local functional adaptation in modern human populations

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Transposable elements (TEs), once regarded as "junk DNA," have recently been recognized for their significant roles in gene regulation and as valuable markers for understanding human evolution. In this study, we explore the functional and evolutionary signatures of polymorphic TEs in modern human populations, with a focus on gene-associated TEs identified in ancient and archaic human genomes. To achieve this, we analyzed whole genome sequences from eight ancient and archaic Eurasian human samples covering the last 80.000 years of human evolution and with a coverage of 4-42X. MELT and TypeTE softwares were employed to identify both reference and non-reference TEs belonging to the Alu, LINE, and SVA families. Following standard quality control, TEs present in at least one ancient/archaic individual were searched within 20 non-admixed populations from the 1000 Genomes Project, with a frequency greater than 0.05 in at least one modern population. Gene-associated TEs were then examined for local signals of positive selection (rEHH) and adaptive introgression (VolcanoFinder), with additional dating of their emergence in the human lineage using a modified version of the GEVA (Genealogical Estimation of Variant Age) algorithm, implemented for more accurate assessment of TE origin dates and confidence intervals. Functional enrichment and gene network analysis were conducted through STRINGdb, DAVID, and PANTHER. Our results reveal that polymorphic TEs from archaic and ancient human populations significantly influence key biological pathways and phenotypic traits in modern humans, particularly those related to immune function, brain development, mental health, anthropometric traits, and cardiovascular health. In some regions, these effects were geographically restricted, while in others, they exhibited clear clines reflecting known human migration patterns.

Genomic analysis of the Vesuvius victims in Pompeii: the case study of the "Room of the Skeletons"

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The ruins of Pompeii, discovered in the 16th century, have been systematically excavated since 1748, offering invaluable insight into ancient Roman society and yielding exceptional discoveries, such as the "House of the Garden" in Regio V. Excavated in 2018, this domus is renowned for the discovery of a charcoal inscription that revised the date of eruption, as well as for its rich decorative elements and numerous finds. In one of the rooms – referred to as "Room of the Skeletons" – the commingled remains of at least 11 individuals, including adult woman and children, were uncovered.

In this study, we genetically characterized 18 bone and tooth samples to determine the number of victims, their biological profiles, genetic heritage, and familial relationships. Additionally, we analyzed a tooth of the "fugitive" discovered near the House of the Garden. By integrating paleogenomic analysis with forensic methodologies, archaeological context, and anthropological assessments, we identified 10 individuals: three adult women and six subadults who sought refuges in the room, and the male who perished while attempting to escape from the eruptive fury of Vesuvius. Kinship analysis revealed varying degrees of relatedness among the victims.

Population genomic analysis demonstrated high genetic diversity within the group, with unrelated individuals exhibiting distinct mitochondrial and Y-chromosome haplotypes and diverse genetic ancestries. Notably, a mother and daughter pair showed an unexpectedly high genetic distance, likely attributable to paternal lineage.

Finally, pigmentation traits and functionally relevant SNPs were analyzed to infer phenotypic features and potential genetic disorders of the victims.

Genetic Profile of the Etruscans from Felsina: genetic changes and Biological Interactions with Neighboring Populations from the Villanovan to the Imperial Period

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During the transitional phase between the Late Bronze Age and the early Iron Age the Villanovan culture emerged in central Italy, within the regions that would later constitute the territory of the Etruscan civilization. In this context, Felsina (modern-day Bologna), part of the northern expansion into the Po Valley, was key for the Etruscan population, as a major center of cultural and commercial exchange during the Iron Age.

Genomic studies on Etruscan-associated human remains from central Italy show that the Etruscans carried a local genetic profile, with only a few individuals displaying ancestry associated with Central European populations. In addition, archaeological evidence points to limited interactions between the Etruscan communities of Felsina and culturally Celtic-related groups during the Hallstatt period (XIII-V centuries BCE), before the historically documented Gallic expansion during the La Tène period (VI-I centuries BCE). To shed light on the genetic relationship between the Etruscan population, the contemporary Italic population and transalpine groups, this study focused on the genomic analysis of 77 ancient individuals excavated from 10 archaeological sites in Bologna, dating from the 9th century BCE to the 3rd century AD.

Genome-wide data were generated, and enriched using a capture protocol targeting approximately 1.24 million informative nuclear SNPs. Individuals with sufficient coverage and low contamination levels were included in population genetic analyses: Principal Component Analysis (PCA), ADMIXTURE modeling, F-statistics, qpAdm admixture modeling, and community structure analysis based on Identical-by-Descent (IBD) segments.

The majority of Etruscan samples from the Po Valley exhibit a genetic profile consistent with local ancestry, in line with patterns observed across the Italian Peninsula during the Bronze and Iron Ages. However, 17% of individuals show a non-local genetic component, associated with Central and Northern European populations, and display increased affinity to steppe-related ancestry. The cluster of individuals showing the closest affinity to Central-Northern European groups stands out as genetically distinct from the local variation, further supporting the hypothesis of gene flow and biological interactions between Felsina and Celtic-related communities. This genetic signal is detected in individuals as old as the 8th, suggesting extensive contacts between them starting at least by the Hallstatt period.

Diet and Lifestyle in the Bronze Age through the Lens of the Oral Microbiome: The Case study of “Terramare” culture

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The European Bronze Age presents a cultural puzzle, situated between widespread patterns and highly localized expressions1. While regional differences in ceramics and metalwork have received much scholarly focus, aspects like subsistence strategies remain less explored. It’s often assumed that Bronze Age diets continued Neolithic practices, with little change in the use of plants and animals. Discussions on food typically relate to broader questions of social structure, such as age, gender, or inequality. In this period more than others, food practices are often seen as reflecting or reinforcing social boundaries. In Italy, late prehistoric dietary studies highlight notable regional differences, but efforts to examine variations across social groups have yielded limited results.

Recent research has shown that the dental calculus microbiome is an exceptional source of information on diet, health, and lifestyle. Emerging evidence suggests that shifts in culture and diet—from hunter-gatherer to Neolithic societies can be traced through oral microbiome. This study aims to investigate through metagenomic analysis the composition of the oral microbiome of three populations from the Terramare culture and combine these data with stable isotopes from bone collagen. These results will be compared with data from earlier hunter-gatherer, Neolithic, and contemporary Bronze Age societies. The objectives are to: (i) assess whether dietary habits reflect continuity or change between the Neolithic and the Bronze Age; (ii) identify distinguishing features of the Terramare populations compared to other Bronze Age groups; and (iii) explore aspects of social structure through insights into diet and health.

We collected 70 dental calculus samples from four archaeological sites in Northeastern Italy. The samples underwent shotgun sequencing and were analysed for their metagenomic profiles following specific authentication criteria6. Samples were also analysed through stable isotope analysis to correlate dietary information with oral bacteria. Preliminary results already highlight the presence of a distinctive oral microbiome community in Bronze Age populations, with further differences observed even within “Terramare” groups. These findings underscore the importance of combining complementary biomolecular, isotopic, and archaeological analyses to gain a more comprehensive view of intra-population lifestyle diversity.

Life and death in an ancient female monastic community: the case study of a mummified nun from Fara in Sabina, central Italy (17th century).

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The municipality of Fara in Sabina (Rieti), hosts an important monastic community of Franciscan nuns dating back to the 17th century that discloses a unique collection of 18 naturally mummified and remarkably well-preserved nun remains. These offer a rare opportunity to explore the lives, health and ancestry of women from different social backgrounds who lived in strict seclusion. In particular, the genetic analysis of one of these nuns, identified as "Fara 9", is the subject of this study. In order to proceed with the genetic analysis, a tooth (pulp and tartar) and a calcific pulmonary nodule were sampled.

The preliminary sequencing screening showed a remarkable endogenous DNA conservation. Due to these promising results, we performed deep sequencing of the genomic libraries derived from the nodule, generating approximately 50 million reads and a coverage of 0.5X on the nuclear genome. Sex assignment confirms the individual was a female. A mitochondrial DNA consensus sequence was generated using Schmutzi (mean coverage: 181.5X), allowing assignment to the haplogroup H1a3a. This lineage is predominantly found in present-day populations of Germany and Norway, with the earliest known specimens dating to the 10th–13th centuries CE and retrieved in Norway. The analysis of the mitochondrial DNA also highlighted the genetic predisposition to various pathogenic mutations, including bullous pemphigoid, an autoimmune pruritic skin disease. Its presence could also be supported by leg lesions that were covered with medical bandages prepared according to the traditional monastic pharmacopoeia, which persisted in the mummified remains. The presence of Parvovirus B19, the virus responsible for the childhood rash erythema infectiosum (fifth disease), was also detected and ascribed to genotype 3, regarded as ancestral to the currently circulating genotype 1. Finally, further in-depth analyses will allow for the assessment of whether the calcified pulmonary nodule may be associated with an infection by Mycobacterium tuberculosis.

The individual "Fara 9" is just the starting point of a multidisciplinary project on the whole ancient monastic community, which represents an invaluable biological archive. By combining genetic and bioanthropological data, this research will provide insights into past health conditions, pathogen evolution, and human adaptation, and it will help reconstruct the personal and collective histories of these nuns, their kinship, and genetic legacies.

Genetic Insights into Burial Practices and Population Dynamics at the Punic-Roman Necropolis of Ortacesus (SU, Sardinia)

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The Punic-Roman period was a time of significant change and increasing interregional contact, due to the growing influence of Carthage and the shift in political and cultural domination that occurred with the Roman conquest of Sardinia. The necropolis of Mitza de Siddi (Ortacesus), a rural burial site containing approximately 150 tombs dated to the 3rd century BCE – 4th century CE, was excavated by the Superintendence for Cagliari and Oristano between 1994 and 2005. The site features varied tomb types and both inhumation and cremation practices. While a portion of the grave goods has been published, little is known about the kinship networks represented within the necropolis. It also remains unclear whether the diversity in burial modalities and tomb architecture reflects the coexistence of culturally distinct groups of different ancestries.

Through a research agreement between the Superintendence and the University of Cagliari, our research team has recently resumed the study of the necropolis, conducting a systematic analysis of both the associated material culture and anthropological data from inhumation contexts. While traditional archaeological and osteological methods have provided valuable insights into burial practices, genetic analysis offers a more direct approach to understanding kinship relations, population structure, and admixture patterns. We thus applied aDNA analysis to 36 specimens, preferentially petrous bones, with teeth as a second option. To explore the biological basis of the funerary rite, individuals were selected based on burial proximity, evidence of sequential use of burial shafts, architectural anomalies, and the presence or absence of grave goods.

DNA has been extracted from 19 individuals in a clean lab following strict aDNA protocols optimized for highly fragmented molecules. Molecular sex determination was possible for 16 individuals, aiding our understanding of the funerary rituals. Currently, results confirm a lack of distinction between sexes in funerary treatment. Ongoing experimental steps include target enrichment of mitochondrial and nuclear markers.

The findings will contribute to discussions on kinship, mobility, and identity in ancient societies, particularly regarding the influence of long-distance contact on local communities. Ultimately, this study offers a nuanced perspective on the social fabric of Ortacesus, enriching our understanding of Punic-Roman burial practices and their social implications.

Crossing the Sea: a paleogenomic perspective on the main Italian islands

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The Mediterranean has always played a key role in the peopling processes and cultural shifts that have shaped Europe over the centuries and millennia; among the areas of particular interest for the study of these dynamics, the large Mediterranean islands offer a privileged context due to their strategic position and abundance of archaeological evidence. However, despite the important role played by these islands, their contribution to the genetic history of the Mediterranean certainly requires further investigation: the extension of the sampling and the time horizon, as well as the increase in the information potential of the genetic data produced, would help to overcome the limitations of the studies already available in the literature.

This is the context for the PRIN2020 “Crossing the Sea” project, which is mainly aimed at reconstructing the genetic history of the main Italian Mediterranean islands from the Upper Palaeolithic onwards, through the analysis of ancient and modern genomic data using a multidisciplinary approach involving archaeologists, anthropologists, palaeoanthropologists and population geneticists.

The paleogenomic analyses involved more than 260 ancient individuals from Sicily and Sardinia, from which 55 were selected so far, according to their molecular preservation, and yielded whole genome data with a mean coverage between 0.1x and 5.3x.

Starting from Sicily, different computational approaches, such as genotype imputation and genotype likelihoods methods, were applied according to the genome coverage. Subsequent analyses including principal component, admixture, IBD and demographic modelling were performed or are in progress.

The results so far produced made it possible to outline the genetic history of Sicily and to compare the evidence obtained with what happened in the past in the Italian peninsula as well as in Europe.

Future analyses will involve the genome data obtained for the Sardinian samples and, in addition, in-depth studies will be carried out on specific sites to investigate local dynamics in a greater detail.

Crossing the Sea: Contextualizing maternal history of Sicily in the Mediterranean. First Results

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Situated at the crossroads of Europe, Africa, and the Near East, Sicily has played a central role in Mediterranean population dynamics for millennia. Here we present the first results from an integrated study of mitochondrial DNA variation in Sicily, combining 41 newly sequenced modern mitogenomes with 19 ancient individuals spanning key prehistoric and historic periods. These data are analyzed in the context of several hundred comparative mitogenomes from Sicily and other regions of the Mediterranean and Europe.

DNA was extracted from teeth and petrous bones and sequenced. The bioinformatic pipeline included consensus sequence extraction, haplogroup assignment, multiple sequence alignment, phylogenetic tree construction, and visualization in R.

Our preliminary analyses reveal both continuity of certain maternal lineages over time and clear signatures of gene flow associated with known demographic events. Characteristic early diverging haplogroups like HV, U5, U8 are associated with early peopling of Europe.

Specifically, several individuals carrying haplogroup HV were linked to the Phoenician culture, such as those discovered in Mozia in western Sicily, while one individual with HV was associated with the Punic culture at Selinunte. Two other HV individuals are modern samples from Agrigento and Trapani, representing the current populations of Sicily.

Additionally, haplogroup U1 was identified in southern Sicily in remains dating to the 1st century BCE, and U2 was found in the San Teodoro Cave, dating back to the Upper Paleolithic. Haplogroup U5 appears in a modern sample from Caltanissetta and other samples dating to the Iron Age and Sicanian periods. Haplogroup H was the most prevalent haplogroup in our overall dataset and one of the most common in Europe, is also found in ancient African samples.

Patterns of haplogroup diversity and phylogeographic affinity highlight the layered nature of Sicily's maternal ancestry, reflecting its long-standing role as a genetic and cultural contact zone in the central Mediterranean.

This study offers new perspectives on the demographic history of Sicily and contributes to broader efforts to reconstruct mobility and admixture across the Mediterranean world through integrative archaeogenetic approaches.

“SHOVELING into the North Italian Bronze Age with an integrative approach to unearth the evolutionary origins of a non-metric dental trait”: a PNRR 2022 project

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We observed in the necropolises of the Terramare (Northern Italian Bronze Age populations, 1859-950 BCE) a large incidence of shovel-shaped incisors, a dental non-metric trait currently common among extant Asians (65%-97.5%) and Native Americans (99.5%), but rather rare in other populations. Tooth shoveling was not uncommon in prehistoric Europe, yet the frequency we found in the Terramare was quite unusual. Different evolutionary explanations can be proposed to explain this phenomenon, keeping in mind that the trait is considered due to a mutation in a pleiotropic gene, EDAR, which might have arisen in China or Beringia about 20000 years ago and was possibly under positive selective pressures: (i) coeval migrations from Asia coupled with demographic events; (ii) independent ancestral inheritance from a hominin; (iii) different mutations in EDAR and selection due to diverse possible agents.

To test the suggested hypotheses, we have proposed an integrative approach which includes anthropological investigation, paleogenomics and stable isotope analyses (C, N, S, O and Sr). We examined and collected samples from individuals with and without shovel-teeth from several Terramare’s necropolises from Veneto (Castello del Tartaro, Bovolone, Franzine Nuove, Olmo di Nogara), plus individuals from other regions and periods. Dental metric and non-metric traits were studied on more than 300 individuals, while more than 350 human samples were sampled for isotope analyses, and 25 individuals were selected for palaeogenomic investigation with target enrichment.

We investigated the genetic basis of dental shoveling by analyzing the rs3827760 variant of the EDAR gene, known for its association with shovel-shaped incisors. Among the 25 ancient individuals analyzed, those for whom genotype data were obtained carried the ancestral allele not associated with the trait, although some showed clear morphological evidence. This suggests additional mechanisms beyond this phenotype. Here, we will show the results of the analyses on selected individuals to highlight from an ancient perspective the evolutionary aspects behind the development of a pleiotropic gene and the interplay with the environment.

Multidisciplinary Characterization of an Etruscan Male with possible DISH from Sasso Marconi (Bologna, Italy), 5th -4th centuries BCE

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The Etruscan civilization and its populations continue to raise questions, particularly regarding their origins, cultural integration, health burden and related medico-surgical practice. In this study, we present a multidisciplinary analysis of the TB2 individual, discovered in 1970 in Sasso Marconi (Bologna, northern Italy) and associated with the Marzabotto necropolis, dated to the 5th–4th century BCE. The skeleton, presently exhibited in the "Pompeo Aria" Etruscan National Museum of Marzabotto and still partially interred, is well-preserved and anthropologically ascribed to a mature/senile male adult.

Initial paleopathological evidence suggests the individual may have been affected by Diffuse Idiopathic Skeletal Hyperostosis (DISH), a diagnosis supported by skeletal features such as ligamentous calcifications and enthesopathies, as well as the presence of an object in the burial that could be interpreted either as a cane or as a scepter. While this may suggest mobility issues, its placement in the grave diverges from traditional Etruscan mortuary symbolism, making it more likely to have been a power insignia, thus indicating a high-status individual. We performed whole-genome sequencing, achieving 1.1x nuclear and 91.1x mitochondrial coverage, with 43.26% endogenous DNA. Using genotype imputation with GLIMPSE v2, we characterized over 42 million SNPs with a mean genotype probability of 99.5%.

These data were used to infer the individual's ancestry and general clinical profile. Given the known correlation between DISH and metabolic disorders such as diabetes and obesity, our focus was on variants related to these conditions. The individual displays a predominantly heterozygous genotype at loci potentially associated with DISH in genes: COL6A1, FGF2, BMP4 and PPP2R2D. As the skeleton remains partially embedded in burial sediments, X-ray imaging will be performed to support the paleopathological diagnosis, while stable isotope analysis (^{13}C and ^{1}N) is programmed to investigate dietary habits. A protein- and fat-rich diet, if confirmed, may constitute a supportive element in the diagnostic process of the suspected pathology as well as be consistent with the elevated social status suggested by the burial context. This integrated, transdisciplinary approach provides a valuable glimpse into the life history and health condition of an elite Etruscan individual and outlines a step-by-step framework for the comprehensive characterization of ancient humans.

Integrating bioarchaeology and paleogenomics to investigate early-life stress in Leopoli-Cencelle (RM, 9th–16th C.E.).

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The study of childhood in past populations has recently expanded, particularly in relation to the dissection of the harsh living conditions triggering the high infant mortality rates, such as high population density, poor hygiene, and repeated exposure to stressors. As for many complex traits, the genetic liability to certain anthropometric characteristics could be impacted by those detrimental environmental factors, limiting the expression of genetically regulated phenotypes. This project investigates childhood adversity by integrating skeletal and genomic data from the medieval cemetery of Leopoli-Cencelle (Rome, 9th–16th C.E.), notable for its high proportion of non-adult individuals (38% of 877 burials). A representative sample of 85 child skeletons (aged 0–19 years) was selected based on the preservation status and distribution across age groups. Macroscopic analysis focused on growth patterns and skeletal stress markers detected either by osteological and imaging analyses to identify signs of fractures, dysmorphic changes, metabolic disorders, congenital anomalies, and deficiency-related conditions, thereby enhancing both the accuracy of health and developmental assessment and providing information on biomechanical stress. Ancient DNA (aDNA) was extracted from the petrous bones and teeth of nearly 70 selected samples. The sequencing results showed good endogenous content in multiple cases. By focusing on the whole genome variability, the detailed osteobiographies, and leveraging reference data from large present-day cohort such as UK Biobank, we will analyse the SNP heritability and association models of multiple phenotypes related to the biological consequences of childhood stress in order to identify risk loci impacting on the childhood resiliency in ancient time.

The research was financed by MUR "PRIN 2022 - Prot. 202255L4YW", "Early-life adversities: writing a biological history of childhood through a transdisciplinary approach" and the financial support of Regione Lazio through ISIS@MACH facilities.

Echoes of the Goths: Archaeological, Anthropological and Biomolecular Insights from two Northern Italian communities during the 4th-6th century CE

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The Migration Period saw the arrival and settling within the Roman Empire of numerous "barbaric" groups. Several territories in Northern Italy experienced significant cultural and political changes, including those gravitating around the ancient Po River delta, due to their strategic location that facilitated defense and movement of people and goods. The demographic impact of these events, however, is unclear and has long been debated.

The Goths, particularly the Ostrogoths, played a crucial role in this transformation. After the fall of the Western Roman Empire, the Ostrogoths, led by Theodoric the Great, established a kingdom in Italy. This period saw a fascinating blend of Gothic and Roman cultures, with the Goths adopting many aspects of Roman administration and lifestyle.

Archaeological evidence, such as grave goods and settlement patterns, provides insights into the ethnocultural identities of these groups. For instance, findings from cemeteries in Northern Italy reveal a blend of Gothic and local traditions, indicating a degree of cultural assimilation.

The sites of Chiesazza di Ficarolo and Chiunsano (Rovigo, Italy), dated between the 4th and 6th centuries, may be an example of this integration. Excavations at these settlements, located between the two main branches of the Po River with direct access to the hydrographic network and land routes, uncovered inhumation burials that represent the first evidence of the Goths' arrival in the Polesine territories. Anthropological analysis was conducted on 56 inhumations from Chiunsano and Chiesazza di Ficarolo. These burials are modest, lacking grave goods, and the individuals are oriented east-west in a typically Christian manner. However, there are intriguing exceptions, such as two unique burials with rich Ostrogothic grave goods at Chiunsano and a group of deviant burials at Chiesazza di Ficarolo.

Twenty-four individuals have been sampled for biomolecular analysis, currently ongoing at the aDNA laboratory of the University of Mainz.

In this presentation, we aim to share the results of the anthropological investigation on the population composition and health conditions, and preliminary findings from the biomolecular analysis.

Biomolecular analysis will help in identifying the ancestry of the individuals and understanding demographic and population dynamics during this historical period marked by the settlement of barbarian communities in these territories.

Sessione

Antropologia Molecolare

poster

Piazza d'Armi of Spoleto (PG, 720 – 580 BCE): a bioarcheological research to understand the Umbrians origins and their complex social relationships

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Among the pre-Roman populations of Central Italy, research on the Umbrians is limited when compared to the coeval and geographically close Etruscans and Picentes. The Iron age necropolis of Piazza d'Armi at Spoleto (PG), dated to the Orientalizing period (720 – 580 BCE) represents an ideal context to try to fill this gap in bioarchaeological studies. This site is unique for the extraordinary richness of its burials. Weapons and sceptres associated with infants and perinatal individuals, both male and female, have been interpreted as a proof of the first attempt to transfer social status and power across generations. Furthermore, some grave goods found in female burials may indicate their involvement in priestly roles. This hypothesis opens new perspectives on the role of women and children in that community, an issue that remains often obscure in reconstructions of early Italic societies.

The Spoleto necropolis is a complex context not only for the luxury of the grave goods but also for the structure of its society, rising questions on whether the elite was formed by a singular aristocratic nucleus or more, as suggested by the numerous round barrows, and whether it was formed by local people or also foreigners, considering the presence of non-local objects.

To test these hypotheses and to further investigate Umbrians' society through genetic ancestry, mobility pattern, and diet, a multidisciplinary approach was adopted focusing on the analysis of ancient biomolecules (DNA and stable isotopes). Skeletal remains were collected and studied from 38 individuals. DNA was extracted from teeth and petrous bones and then converted into double-stranded genomic libraries. Whole-genome shotgun sequencing and DNA capture with "Twist Ancient DNA" assay targeting over 1.2M SNPs were performed. Specific bioinformatics aDNA pipelines were employed to authenticate sequences, assess individual ancestry, and reconstruct genetic kinship.

Migration pathways were investigated by comparing Sr isotopic signature from tooth enamel, bones and soil. Dietary reconstruction was based on C and N isotopic ratios from collagen which reflect the isotopic signature of foods consumed in the last years of life. Both analyses were carried out using mass spectrometry.

These data provide preliminary information about the origins of ancient Umbrians, their social structure and relationships with the other pre-Roman populations.

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Preliminary paleogenomic analysis on individuals associated with the Villanovan culture in Northern Italy

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The origin of Etruscan civilization is still the subject of much debate today. Two main hypotheses have been debated over time: one suggesting an eastern origin (Anatolian or Aegean) and a second supporting an allochthonous origin from the Villanovan culture, which flourished in central Italy between the 9th and 8th centuries BC. Even if recent studies pointed to a local origin of the Etruscans, no direct genetic comparison between Villanovan and Etruscan individuals has been carried out to date, mainly due to the poor preservation of genetic material in cremated skeletal remains, a common funerary practice in Villanovan culture. In this context, the analysis of ancient DNA from individuals of the Villanovan period is crucial to better understand how and where the Etruscan civilization developed.

Here we present the preliminary paleogenetic results on a set of 24 individuals from three necropolis in the area of Bologna archaeologically dated between the 9th and the 8th–7th centuries BCE. The necropolis of San Vitale, Benacci, e Arnoaldi were excavated between the end of the 19th and the beginning of 20th centuries and the rich funerary materials recovered became part of the collections of the recently established Museo Civico Archeologico di Bologna. For this work we selected petrous bones from inhumated individuals that were analyzed using laboratory techniques specifically made for the study of ancient DNA. Subsequently, specific bioinformatics tools were used to assess DNA preservation, estimate modern DNA contamination, and to perform preliminary genetic analyses.

The samples exhibited high molecular preservation with endogenous DNA percentage ranging between 20 and 88%. Molecular sex determination, reconstruction of uniparental markers and the determination of kinship relationships among individuals were performed. In order to confirm the archaeological chronology, a subset of samples were radiocarbon dated. The analyzed individuals carry a genetic profile which seems to resemble the one previously found in Etruria and, overall, in the rest of the Italian Peninsula during the Iron Age (and the Bronze Age),

Considering the informative potential of the samples here analyzed, additional work will involve more informative population genetic analyses in order explore more in-depth the ancient phases of the development of the Etruscan Population, as well as their relationships with contemporary and earlier groups from central Italy.

Ancient DNA Methods Meet Forensic Science: Sex Estimation from Hair Evidence

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Sex estimation is a fundamental step in forensic anthropology for constructing a reliable biological profile from skeletal, and it becomes especially critical when dealing with hair samples. In such cases, traditional STR-based methods are often not applicable due to the high degradation of nuclear DNA. Next-generation sequencing (NGS) offers a powerful alternative, enabling sex determination even from highly degraded samples.

Among the most promising NGS-based approaches are the Ry and Rx methods, which infer sex by analyzing the proportion of DNA reads aligning to the X and Y chromosomes. Originally developed for ancient DNA, these methods have shown high accuracy in anthropological/archaeological contexts, but their forensic applicability - particularly to hair samples-remains underexplored.

This study aims to evaluate the Ry and Rx methods for forensic use in hair-derived samples, where they represent the only viable strategy for sex estimation. To this end, we tested the methods on unique twenty-eight samples high-coverage genomes (˜40X) from the 1000 Genomes Project (16 females, 12 males) with known sex. At high coverage, both methods showed complete concordance with the known sex, confirming their accuracy.

To simulate forensic conditions, we artificially degraded the genomes using bioinformatic tools, downsampling to 30x, 20x, 10x, and 5x, and introducing 15%, 10%, 5% and 1% damage using Gargammel software. Additionally, we are refining the analytical pipeline to assess performance under increasing levels of contamination (10%, 25%, 50% e 60%). This work represents a foundational step toward implementing NGS-based sex estimation in real forensic casework involving hair, where traditional methods fail and NGS remains the only viable solution. Validating these approaches under controlled conditions is essential to ensure their reliability and applicability in real-world forensic investigations.

APOE DNA methylation variability in human populations and its implication for human health

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Apolipoprotein E (APOE) haplotypes are among the most extensively studied genetic factors associated with age-related diseases, human longevity and fertility in natural fertility populations. APOE isoforms present variability across different populations as well as high pleiotropic effects. However, despite the strong associations with phenotype identified, the geographic distribution of the genetic variants does not always reflect the distribution of these traits in human populations, suggesting complex gene-environment interactions. Among the many biological mechanisms beyond, DNA methylation may certainly play a major role. Despite the high number of studies on genetic variations, few studies that mainly focused on populations of European ancestry have investigated the role of DNA methylation at APOE CpG sites. Moreover, the interactions between APOE genetic variants and DNA methylation variation in non-European populations and in natural fertility populations are still missing.

In this framework, the aim of this study is to investigate:

1. patterns of natural variations of 11 CpGs sites located in the APOE in non-European populations;
2. the interactions between APOE genetic variants and DNA methylation variation in non-European populations

To this aim we considered individuals from different ethnic groups of global distribution whose methylation data are publicly available, including those of indigenous Latin Americans, as well as native and admixed populations of Argentina, such as the Criollos and Wichí. The latter populations represent a unique context for studying genetic diversity and epigenetic variability for which genotypic data further allows to investigate genotype-specific methylation patterns. We used data from the Infinium Human Methylation 450 array and the Infinium MethylationEPIC BeadChip, for public data, those from online databases, mainly the Gene Expression Omnibus (GEO). The results showed natural variations among human groups in DNA methylation patterns in a subset of CpGs sites. Moreover, the study provides new information and results on the association between DNA methylation and APOE isoforms.

In conclusion, we present preliminary insights into the DNA methylation variability associated with the APOE gene and its isoforms, which is crucial for further investigations into the role of methylation in modulating the effects of APOE genetic variants across different environmental, ecological, and anthropological contexts.

Gene Polymorphisms for Strength and Sprint Performance among Female Volleyball Players.

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Background. Volleyball is a high-performance intermittent sport that requires alternating aerobic and anaerobic phases, with anaerobic alactic metabolism used for explosive efforts.

The ACTN3 rs1815739, MCT1 rs1049434 and ACE I/D polymorphism are the most studied genetic markers in sport sciences, and they have been associated with sprint and strength performance. ACTN3 codes for the -actinin-3 protein, which is a decisive factor in the ability to produce high-velocity muscular contractions. MCT1 codes for monocarboxylate transporters 1 that plays a relevant role in the intracellular pH homeostasis. ACE code for angiotensin converting enzyme, that has a relevant role in fluid homeostasis.

Aim of the study. This study examined the distribution of common genetic variants in the ACTN3 (rs1815739), MCT1 (rs1049434) and ACE (I/D) gene and their influence on short linear sprint and strength performance in female volleyball players.

Methods. A total of 99 female volleyball players (Elite, sub-elite, and amatorial) participated in the study. In a sub-sample of 66 athletes (sub-elite and amateurs) some anthropometric variables and motor tests were measured. 3 SNPs in ACTN3, MCT1, and ACE were performed.

Results. No significant differences have been found in allelic frequency distribution among the 3 groups of athletes. The sample met the Hardy Weinberg equilibrium for the 3 polymorphisms. The allelic frequencies of ACTN3 and MCT1 (ACTN3*R= 0.5885, ACTN3*X= 0.4115; MCT1*A= 0.3594, MCT1*T= 0.6406) are in the range of worldwide population variability, on the contrary ACE I/D (ACE*I= 0.3670, ACE*D= 0.6330) showed an increase of D frequency.

The association analysis, carried out through SNPSTAT program, gave back significant results only for ACTN3/5m sprint. Precisely, the players harboring a copy of the R allele (RR+RX genotypes) showed a better sprint time than players with XX genotype (RR+RX= 1.27±0.01 sec vs XX=1.36±0.04 sec, p=0.023). Differences were also found among positions on the field, with liberos that were significantly fastest than setters (p = 0.021) and middle blockers (p = 0.010).

Conclusion. We found, for the first time, an association between the ACTN3 polymorphism and liner running speed in youth female volleyball players. If our findings are replicated in larger research, it could be used in future to adapt the training protocols to enhance volleyball performance.

Urban Kinship and Genetic Diversity in Thessaloniki: A Genomic Perspective

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Urbanization has long fostered interaction and interdependence among individuals beyond close biological kin. Yet, we still know little about how familial, religious, and economic networks shaped everyday life in Roman and post-Roman urban centers. This study employs ancient DNA analysis to investigate such dynamics in the city of Thessaloniki — a major urban and cultural hub throughout European history. Our dataset spans the entire Byzantine period (324–1453 CE), with a particular emphasis on the Late Byzantine period (1204–1453 CE).

We explore how the formation of social, religious, and neighborhood-based subgroups may have encouraged endogamy, thereby reducing genetic diversity within subgroups. At the same time, factors such as trade, migration, and exogamous marriage practices likely contributed to increased genetic diversity at the population level.

In addition to standard approaches for inferring ancestry and biological relatedness, we applied population genetic metrics including inbreeding coefficients (F), Wright's FST and nucleotide diversity estimates (and) to assess genetic structure and heterogeneity. We integrated these genomic findings with historical and archaeological sources to better understand how cultural norms and social organization influenced patterns of diversity in the city.

Our results indicate clear signs of population structure especially in Byzantine Thessaloniki, which we interpret as a probable reflection of localized family systems and broader socio-cultural frameworks.

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Untangling the complex hybridization history of true lemurs (genus Eulemur)

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The true lemurs (genus Eulemur) are a genetically diverse and geographically widespread group inhabiting most of Madagascar's forests. With 12 recognized species, the genus comprises four major clades: the Brown Lemur Species Complex (BLSC), the coronatus-macaco-flavifrons complex (CMFC), E. mongoz, and E. rubriventer, although monophyly for the CMFC is not always supported. Recent genomic studies revealed topological and temporal discordances between nuclear and mitochondrial phylogenies in Eulemur, likely due to past hybridization events. To investigate the evolutionary history of the genus, we compiled a dataset of previously published whole-genome sequences and reconstructed both nuclear and mitochondrial phylogenies. Using this framework, we applied a population genomics approach (ABBA-BABA tests, hPSMC, and admixture graphs) to test for gene flow among clades. We detected multiple hybridization events, most notably between E. rubriventer and the BLSC, helping to explain mito-nuclear phylogenetic discordance. To further explore this, we examined nuclear-encoded mitochondrial proteins in E. rubriventer and found an enrichment of topologies matching mitochondrial phylogenies, suggesting mito-nuclear co-adaptation following introgression.By directly testing for hybridization across the genus, our study presents a refined evolutionary model for Eulemur, highlighting the role of gene flow in shaping its genomic landscape, while also agreeing with the most supported ecological model for the rapid speciation of the genus during the last 5 million years. These findings contribute to a deeper understanding of this emblematic Malagasy lineage and lay the groundwork for future research.

Kinship Reconstruction of the Velimna Family from the Hypogeum of the Volumnis (3rd Century BCE, Umbria)

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The genetic history of central Italy remains only partially explored. While several genomic studies have shed light on ancient groups such as the Etruscans in Tuscany and the Picenes in Marche, the region of Umbria and the ancient Umbrian population has yet to be fully investigated.

In this study, we aim to investigate the genetic history of the Volumnia family buried in the Hypogeum of the Volumnis, within the context of the Necropolis of Palazzone (III BCE), a monumental tomb that preserves the urns of seven members of the Velimna family. Inscriptions at the site suggest close familial relationships among six of them, offering a unique opportunity to reconstruct biological relatedness using ancient DNA.

The samples analyzed included three cremated individuals and one inhumed individual (Velia Velimnei).

DNA was extracted from highly degraded and carbonized petrous bone fragments in a dedicated clean lab following strict aDNA procedures.

Genomic libraries were prepared using methods optimized for highly fragmented molecules. Shotgun sequencing in combination with a target enrichment approach were used to reconstruct mitochondrial and nuclear genome of the analyzed individuals.

The shallow shotgun sequencing enabled assessment of endogenous DNA content and contamination levels, guiding the application of whole-genome deep sequencing for well-preserved samples and targeted enrichment for those with lower endogenous DNA content.

The authenticity of aDNA is evaluated through the damage patterns analysis and by examining the heterozygosity of the X chromosome in the male individuals.

Biological sex was inferred using the ratio of reads aligning to the sex chromosomes.

Finally, the phenotypically and functional informative SNPs will be analyzed for well-preserved individuals.

This study will thus allow for a deeper investigation of the genetic relationships among the members of the Velimna family.

Uncovering Genetic Signatures of an Iron Age Umbrian Population from Montecchio, Necropoli del Vallone di San Lorenzo (Umbria, Italy)

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The complex demographic and cultural transitions that occurred during the Iron Age which shaped pre-Roman Italy still remain a central topic in molecular anthropology. During the Iron Age (c. 1000–350 BCE), before the unification under the Roman control, the Italian Peninsula is populated by several regional groups with different cultural identities and different languages which are traditionally called “The Italic peoples”. In this study, we present preliminary genetic data from eight human skeletal remains excavated at the necropoli del Vallone di San Lorenzo, Montecchio (Umbria, Central Italy), dated to the late Iron Age (ca. 6th to 5th centuries BCE). The archaeological context, including artifactual evidence and tomb typology, points to an Umbrian site with strong Etruscan cultural influence and even possible Picene connections.

Recent ancient DNA studies on Italic people such as the Etruscans and Picenes have revealed a heterogeneous genetic landscape, reflecting both continuity from Bronze Age populations and interactions with incoming groups during the Iron Age.

All eight individuals were processed following the standard ancient DNA laboratory workflow, and the obtained libraries were screened by NextSeq 550 (Illumina) at ISIS@MACH facility, Tor Vergata University. Three individuals were excluded from further analyses due to low endogenous DNA content. The remaining five samples exhibited typical aDNA features, including cytosine deamination (C¿T) at read ends, negligible mitochondrial contamination, and, for males, low X-chromosome contamination as assessed using an optimized custom pipeline. Notably, two samples yielded exceptionally high endogenous content (¿60%). These findings indicate favorable preservation conditions at the site, despite the typically warm climate of central Italy. They also demonstrate the site’s strong potential for generating high-quality ancient DNA suitable for low coverage whole-genome sequencing analyses.

Our long-term objective is to increase genome sequencing coverage at least 0.1 X to enable robust placement of these individuals within the genetic variability of prehistoric Italian populations. Comparative analyses with published data on Etruscan and Picene groups will help to clarify whether the Montecchio individuals share affinities with known Etruscan gene pools, supporting the hypothesis of cultural and genetic continuity in the region during the final stages of the Iron Age, on the eve of Roman expansion.

The origin of social inequalities in Northern Italy: clues from ancient genomes

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Understanding the dynamics that promoted the onset and perpetuation of social inequalities in prehistoric societies is a major challenge. Thanks to the increase in genomic data from ancient remains, the development of kinship estimation methods, and computational approaches for reconstructing past evolutionary dynamics from low coverage data, we now have a unique opportunity to exploit genomic data to describe past population structures and shed light on the socioeconomic processes and sociocultural dynamics underlying the emergence of inequalities. Archaeogenetic has revealed that two major population dynamics in the last 10,000 years significantly impacted the genomic composition of Europeans: the Neolithic expansion and the Bronze-Age migration from the Steppe. Despite the importance of these events, our genetic understanding is mainly built upon pan-European sampling strategies, resulting in limited knowledge of how these migrations affected individual societies. This project proposes a high-resolution multidisciplinary study of approximately 120 individuals from three necropolises in north-eastern Italy dating back to the Neolithic (Valdaro Paganella), Eneolithic (Corna Nibbia di Bione) and Bronze Age (Arano) periods, with the specific aim to infer the social and genetic structures and their potential changes over this time span. We are analysing whole genomes to reconstruct biological relatedness within each necropolis, infer population structure and admixture proportions, and model past demographic dynamics. Currently, we have an almost exhaustive paleogenomic overview of the Eneolithic site of Corna Nibbia. Analyses of the Arano site are providing extensive data, allowing us to draft a preliminary pedigree. Meanwhile, genomic analyses of the Neolithic site are in progress. The genomic data, combined with a high-resolution chronology, archaeological evidence, and ongoing stable isotopes analyses to investigate dietary patterns and individual mobility, will allow us to obtain information about potential inequalities between members of each burial site and to recognise possible patterns behind the transmission of social status and wealth. This multidisciplinary strategy will help us to better understand social inequality in north-eastern Italy, as well as the cultural and biological mechanisms that promoted its development.

Tracing Genetic Heritage in the South Caucasus: Ancient DNA from Bronze Age Armenians

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Armenia has been a key geographical region with intense migratory flows since the Upper Palaeolithic. Due to its proximity to Anatolia, it was among the first regions to adopt agriculture. Another major event was the migration from the Caucasus to the steppe, which contributed to the formation of Steppe ancestry. During the Bronze Age, Armenia as a crossroads between the Middle East, the Caucasus, Central Asia and Iran, become a hub of cultural and linguistic exchange. This complex historical background makes the study of Armenian demographic history essential for understanding regional population dynamics and migration patterns.

Our aim is to genetically analyze 51 individuals from the western and southern shores of Lake Sevan, at altitudes of 1,800–2,000 meters, from five collective burials dating from 2000–1400 BCE. Our principal goals is to characterize this population at genetic point of view and assess potential kinship relationships among individuals buried in collective graves.

Specifically, teeth and petrous bones from five sites: Kanageh, Noratus, Nerkin Getashen, Lchashen and Zorats Karer were analysed. From each sample, a minimum of 50 mg of bone powder was used for DNA extraction, double-stranded libraries were constructed, indexed and screened using the Illumina NextSeq550 at ISIS@MACH facility, Tor Vergata University.

Initial results show good DNA preservation, with endogenous DNA content ranging from 20% to 75%, except in a few cases. Deamination rates (C to T transition) at the 5' end, confirming the ancient origin of the material. The data was authenticated by the mtDNA contamination test using contamMix, and in males the X chromosome contamination rate using ANGSD.

Our screening results indicate good preservation of the analyzed samples, likely due to the low temperatures at the lake's altitude. Notably, this preservation is particularly impressive given that most of the processed samples were teeth, which are generally not considered the most optimal material for ancient DNA analysis in prehistoric contexts. Next step will be achieve a coverage more than 0.5X, sufficient for genotype imputation. These imputed data will be analyzed alongside previously published genomes to enhance our understanding of genetic variability in the Armenian population during the Bronze Age. Furthermore, our analysis aims to elucidate the role of Lake Sevan in the migration routes connecting the Caucasus, northern Levant, Iran, and Anatolia.

PRIN2020 “Crossing the Sea”: Depicting the adaptive evolutionary history of Sicilian ancestors through the analysis of modern genomes

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The Mediterranean Sea played a crucial role during the principal phases of the peopling of European territories and in the major cultural changes associated with them. In this context, Sicily has long represented a pivotal crossroad, acting as a fundamental meeting place for several populations during all the migration processes that interested the continent through time. In addition to such a complex demographic history, the genetic background of present-day inhabitants of the island might have been shaped also by the evolution of biological adaptations in response to local selective pressures, which were plausibly different with respect to those experienced by human groups distributed along the peninsula. While previous studies already addressed the genetic history of Sicilian people, they relied solely on the analysis of uniparental markers or autosomal loci typed with SNP-chip approaches, thus failing to investigate its full spectrum of variation. This also prevented exhaustive identification of the genetic determinants of possible adaptive traits. Within the framework of the PRIN2020 “Crossing the Sea” project, which is aimed at investigating the evolutionary history of populations from the main Western Mediterranean islands, we generated high-coverage whole-genome sequence data for 41 individuals three generations native of different areas of Sicily. Population structure and haplotypes sharing-based analyses setting the examined samples into the context of a large panel of Euro-Mediterranean genomes made up of nearly 900 individuals from 17 populations, revealed that Sicilian individuals grouped within the same genetic cluster and showed proportions of ancestry components appreciably distinct with respect to those observed in peninsular Italians. We thus considered this homogeneous population sample to infer the adaptive evolution of Sicilian ancestors. For this purpose, we applied likelihood- and network-based methods suitable to detect both strong and weak selective events and we searched for biological functions enriched for putative adaptive loci. This approach enabled to test different models of adaptive evolution, ranging from classical selective sweeps to polygenic adaptations, thus providing an unprecedented overview of the environmental factors having represented remarkable selective pressures during the evolutionary history of Sicilian peoples.

Sessione

Biologia ed Ecologia umana

comunicazioni orali

Very similar or widely variable?

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A recurring theme in the literature on body composition and related diseases is the recommendation to compare each sample to a population-specific reference. This contribution discusses the drawbacks and the potential theoretical and methodological flaws associated with this approach.

A central issue concerns the very concept of 'population', which remains ill-defined in human biology and is often improperly used interchangeably with the terms terms 'ethnicity' or 'race'. Despite authoritative statements highlighting the lack of biological validity of such categories, expressions like 'Caucasians' are still widely employed in the biomedical literature.

When used appropriately - to identify groups sharing specific characteristics such as age, sex, or geographic ancestry - a population-specific reference can be useful, for example when comparing athletes to their peers.

However, in health-related contexts, as is typical for most studies on body composition, relying on population-specific references obscures intergroup variability that should instead be acknowledged and analysed. Notably, population-specific features of body composition may also signal widespread adverse health conditions.

From a diagnostic perspective, comparisons should be made against a standard defined as such because it represents a normative or desirable target. In addition, cut-off values should be chosen based on the underlying statistical distributions. Relying on simplistic thresholds (e.g. 2 SD) in the case of non-normal distributions may result in substantial misclassification.

In conclusion, with the exception of studies aimed at focused comparisons, health-related assessments should be based on standards that represent healthy body composition. In the absence of reliable standards, large international references - which include a wide range of variability and patterns of normality - may be a more appropriate alternative to strictly population-specific references. The distribution of variables should always be considered.

Specific bioelectrical vector reference values for Italian adults: a multicentre study

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Specific bioelectrical impedance vector analysis (*sp* BIVA) is a semi-quantitative approach for the analysis of body composition. *Specific* vectors can be evaluated using tolerance ellipses to compare individual or mean characteristics with those of the reference population or using confidence ellipses to make statistical comparisons between groups. Tolerance ellipses are currently available for Italo-Spanish young adults (up to 30 years), Italian older people (over 65 years), and US young adults. The aim of this multicentre study is to define *specific* bioelectrical reference values in Italian adults aged 30 to 65 years.
A sample of 1049 Italian adults (441 men and 608 women) was examined. The anthropometric variables considered were height, weight, mid-upper arm circumference, waist circumference and calf circumference. According to the *sp* BIVA procedure, the bioelectrical values (resistance and reactance, measured at 50 kHz) were standardised by a correction factor, that considers height and cross sections, in order to calculate: resistivity, Rsp, and reactivity, Xcsp (both expressed in ohm*cm). Impedivity (Zsp, ohm*cm) and phase angle (PhA, in degrees) were then derived. Differences between sexes and with previously published reference groups were evaluated by means of Hotelling T^2 test.
Men and women had a similar mean age (men: 48.8 ± 10.9; women: 50.4 ± 10.2). The *specific* bioelectrical parameters were: Rsp (352.3 ± 55.5 ohmcm), Xcsp (41.8 ± 9.1 ohmcm), Zsp (354.9 ± 55.9 ohmcm), PhA (6.8 ± 1.0°) for men; Rsp (384.9 ± 71.2 ohmcm), Xcsp (40.6 ± 8.9 ohmcm), Zsp (387.1 ± 71.5 ohmcm), PhA (6.0 ± 0.9°) for women. The correlation between Rsp and Xcsp was 0.60 for men and 0.72 for women.
The difference between the mean vectors of the two sexes was significant (T^2: 184, $p<0.001$), with men showing higher phase angle and shorter vector. The differences with the younger Italo-Spanish (men, T^2: 76.7, $p<0.001$; women, T^2: 32.0, $p<0.001$) and older Italian (men, T^2: 120.9, $p<0.001$; women, T^2: 215.1, $p<0.001$) samples were significant, with lower phase angles and longer vectors in groups of increasing age. This trend mirrors the age-related changes of bioelectrical values and body composition.
The tolerance ellipses for the Italian adult population fill a gap in the existing literature and provide new tools for evaluating body composition in adults aged 30-65 years and for comparative analyses.

The effects of exercise training on body composition and quality of life in menopausal women

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Menopause is a natural phase in women's lives, characterized by physiological changes affecting physical and mental health. In this context, physical exercise has been proposed as a potential intervention to alleviate menopause symptoms and improve the quality of life of women in this phase.

This pilot study, part of a larger project, EFiM (physical exercise in menopause), which will last three years, examined the effects of a 12-week physical exercise program on a sample of 18 menopausal women on body composition and quality of life in menopausal women.

Anthropometric data (stature, weight, circumferences, diameters, skinfold thicknesses), bioelectrical impedance analysis, and body plethysmography (BOD POD) were carried out. An ultrasound bone densitometry instrument (OsteoSys – SONOST 3000) was used to assess bone density. Thompson & Gray's (1995) silhouettes were administered to examine the perception of body image. To assess the quality of life and well-being, the participants were administered the MENQOL (Menopause specific quality of life questionnaire), which explores 29 typical symptoms of menopause, to which they had to assign a score from 0 to 6 regarding the perception of the symptom. The handgrip strength test (HGS) and the treadmill stress test were performed to assess the subjects' functional parameters. The physical exercise protocol included two weekly sessions, divided into a part aerobic exercise and a part resistance exercise, for 12 weeks. Differences between pre-and post-intervention data of physical activity were assessed by the t-test for dependent samples.

A trend towards the amelioration of body composition parameters was detected. Significant improvements were recorded in strength, menopause symptoms, and psycho-physical well-being, indicating the positive effect of physical activity.

The menopause period is a critical time for interventions to promote physical activity and an active lifestyle, as women often become less active, aging processes become more apparent, and health threats and chronic diseases begin to emerge. Both initiating and maintaining active behaviours pose significant challenges, making it essential to propose targeting interventions and address the psychosocial factors determining short- and long-term adherence.

The impact of urban and rural environment on young adults anthropometric characteristics: a study on a Northwest Italy University students sample

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In developed countries, the influence of the living environment on physical development remains a debated topic with studies suggesting the benefits of urban settings while others point out the rural as the more optimal. The aim of this study was to investigate differences in anthropometric characteristics among students of the University of Torino (Wellness4Students project) according to the area in which they grew up (urban/rural) and their changes after years spent in an urban setting. A sample of 847 students (18-25 years old) was divided into 8 groups based on gender, area and year of study course: 1st year sample consisted of 123 urban and 123 rural females, 76 urban and 97 rural males; 3rd and 4th years sample consisted of 100 urban and 157 rural females, 80 urban and 91 rural males. Height, weight and waist circumference were collected (ISO 7250.1/2017); Body Mass Index (BMI) and waist to height ratio (WHtR) were calculated; percentages of Fat Mass (FM%), Fat Free Mass (FFM%) and Muscle Mass (MM%) were estimated through Classic Bioelectrical Impedance Vector Analysis (BIVA, BIA 101 Pro-Akern). Statistical analyses (normality tests, independent samples t test) were conducted (IBM SPSS Statistics.29) with significance set at p ¡ 0.05. Results highlighted no differences between urban and rural 1st year females in all the variables, while rural 1st year males showed a significantly lower FM% (-2.2 % ± 0.91, p¡0.05) and a higher MM% (2.1 % ± 0.7, p¡0.01) than the urban counterpart. On the contrary, in the 3rd and 4th years sample no differences were found between urban and rural males, while rural females presented significantly lower values of WHtR (-0.02 ± 0.01 p¡0.05). Even though no significant differences were found among the groups regarding BMI, higher frequencies of overweight/obesity conditions emerged in urban samples compared to the rural counterparts specifically in males (+7% in the 1st years sample and +8% in the 3rd and 4th years sample). In conclusion, this study found more MM%, lower FM% and lower prevalence of overweight/ obesity in rural males of the 1st year compared to urban, although these differences seem to attenuate after a few years spent in an urban context. Rural females instead presented lower values of WHtR in the 3rd and 4th years. These findings suggest that the urban environment can have a significant influence on students' physical condition, and this should be further explored and monitored for primary prevention.

Body composition as a tool for tailoring therapeutic interventions in epilepsy

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Body composition (BC) plays an important role in the distribution and dynamics of drugs, influencing treatment efficacy and the risk of adverse effects. This is particularly relevant in epilepsy, one of the most common neurological disorders, which is treated with drugs characterised by a narrow therapeutic index.

Despite its relevance, BC is generally overlooked in clinical practice, mainly due to the high cost, technological complexity, and invasiveness of reference methods. Bioelectrical impedance analysis (BIA) is a suitable alternative, as it is minimally invasive, inexpensive, and quick to use.

The aim of this interdisciplinary project is to analyse the role of BC in drug distribution in patients with epilepsy, using a longitudinal design and taking into account possible influencing or confounding factors (sex, age, cortisol levels, physical activity, multimorbidity, and the presence of SNPs).

In this contribution, we present the structure and first results of the project.

The sample consisted of 118 adults with epilepsy (53 men and 65 women). Baseline BC was assessed by anthropometry, conventional BIA, and bioelectrical impedance vector analysis (BIVA). The most commonly used antiepileptic drugs were levetiracetam (38% of the sample), lamotrigine (17%), valproic acid (9%), and topiramate (9%). Plasma levels were categorised as subtherapeutic (36.4%), therapeutic (58.3%), or supratherapeutic (5.3%), according to treatment-specific reference ranges. The likelihood that drugs influence body composition was above 50% for Z/H, TBW, and FFM. Levetiracetam and Oxcarbazepine showed a positive association with FFM ($p = 0.028$ and $p = 0.006$, respectively) and TBW ($p = 0.023$ and $p = 0.004$, respectively). The probability that body composition affects plasma drug levels was above 20% for FM, FM%, and TBW%, suggesting that additional variables contribute to this relationship. In conclusion, this study represents a translational application of human biology to pharmacokinetics. Preliminary findings suggest that treatment may influence body composition (FFM and TBW), and body composition (particularly FM%) may influence plasma drug levels. The analysis of additional possible influencing factors will help to refine these associations. Longitudinal analysis will help to reduce interindividual variability and clarify the impact of BC on drug distribution.

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Biologia ed Ecologia umana

poster

Linea C terminations in pathological and healthy subjects

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The terminations of the C line in four types have been studied in various populations. In this work the terminations of the C line in some pathologies were analyzed. The conclusion is that the pathological dermatoglyphics study may be an additional character a possible diagnosis but certainly not a significant diagnostic character.

The effect of arm position on bioimpedance measurements

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Bioelectrical impedance analysis (BIA) is a widely used technique for assessing body composition. Various BIA devices and protocols are commonly applied, and their interchangeability remains a critical issue for both research and practical applications.

This study evaluates the impact of body and arm positioning, and electrode placement, on whole-body and segmental raw bioelectrical values, namely resistance (R, ohm) and reactance (Xc, ohm), measured at 50 kHz.

The study examined a sample of 50 adults (35 men and 15 women). Whole-body and arm resistance and reactance were measured with participants in seven different positions — one supine and six standing — designed to reflect the positions commonly adopted with commercial BIA devices. In the standing positions, the arms were either extended parallel to the body or abducted to 45°, 60° or 90°, or flexed at the elbow to 45° or 90°. Two different electrode placements were used for arm measurements. Differences in R and Xc values across positions were evaluated using ANOVA with Bonferroni post hoc tests.

At the whole body and arm level, R and Xc values measured in the supine position mostly corresponded to those obtained in the standing position with the arms extended parallel to the trunk, in both sexes. R values increased progressively with greater arm abduction and decreased with elbow flexion, particularly at 90°. Xc also varied, though the changes were slightly less pronounced. While most differences were statistically significant, they mostly fell within the acceptable range of device variability (±10 for R and ±5 for Xc for the whole body). However, R values in the 90° flexed position and in the maximally abducted position were outside this range for both sexes. A similar, albeit slightly more pronounced, pattern was observed for whole-body values, where both flexed positions produced R and Xc differences outside the acceptable range. Alternative electrode placements only partially mitigated the effect of arm position.

In conclusion, arm position exerts a greater influence on segmental bioimpedance measurements than body posture, reaching biological relevance when the arm is flexed. Further studies are needed to assess the systematic nature of these differences and thus support the formulation of correction factors.

Phase Angle and bioelectrical impedance vector analysis as Prognostic Indicators of Functional Recovery in Postoperative Femoral Fracture Rehabilitation

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Elderly patients with femoral fractures require targeted rehabilitation after surgery to enhance self-reliance and quality of life, while also reducing mortality. Although poor body composition is associated with worse outcomes, it remains unclear whether bioelectrical impedance analysis (BIA) parameters can reflect or predict functional recovery. To explore the potential of BIA as a biomarker in rehabilitation, a case-crossover longitudinal study was conducted involving 41 elderly patients (mean age 84.6 ± 7.2 years; 75.6% female) who underwent femoral surgery. Body composition (via anthropometry and BIA) and self-reliance were assessed at three time points during follow-up, focusing on both the operated and non-operated limbs.

A two-way repeated-measures ANOVA, adjusted for sex, age, and leg dominance, revealed significant improvements in activities of daily living. Thigh circumference decreased over time regardless of surgical intervention. In terms of BIA parameters, resistance (R) decreased more prominently in the operated leg, while reactance (Xc) showed similar trends in both limbs. Phase angle (PhA) presented differential changes between treated and untreated limbs, and between right and left legs, with a maximum recovery-related reduction of up to 16.8% (95% CI: 3.4–28.4). These findings highlight the need for personalized attention in geriatric rehabilitation. BIA presents a promising, non-invasive tool for monitoring recovery and may help optimize healthcare resource allocation.

Body composition in patients with epilepsy

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Epilepsy is among the most common neurological disorders, affecting over 70 million people worldwide. Variability in body composition (BC) may result from treatment effects and, in turn, influence pharmacokinetics and therapeutic efficacy.
This study analyses BC in 110 Italian adults with epilepsy (46 men, 64 women). Anthropometric data (weight, height, mid-upper arm, waist, and calf circumferences) and bioelectrical measurements (resistance and reactance at 50 kHz) were collected. BC was assessed using conventional bioimpedance analysis, phase angle (PhA) analysis, and both classic and specific bioelectrical impedance vector analysis (BIVA). Participants also completed questionnaires on adherence to the Mediterranean diet (MEDILITE) and physical activity (IPAQ).
Men and women were of similar age (men: 46.5± 20.6 years; women: 46.6 ± 16.2 years; $p = 0.991$). Adherence to the Mediterranean diet was generally good across both sexes. Nearly half of the participants were physically inactive (men: 46.3%; women: 56.1%). The mean BMI (men: 28.5 ± 5.4kg/m^2; women: 26.9 ± 5.7kg/m^2) and fat mass percentage (FM%; men: 28.7 ± 9.8%; women: 37.3 ± 7.4%) indicated overweight in both sexes, based on the thresholds proposed in the literature.
When plotted on classic and specific BIVA tolerance ellipses, over 80% of the vectors were on the right side, with low PhA values indicating reduced muscle mass and low intracellular/extracellular water ratio. Specific BIVA revealed a tendency towards elongated vectors in men and women aged 18-65 years, indicating high FM%. Classic BIVA showed longer vectors in both sexes and in the same age group, indicating a tendency towards dehydration.

In conclusion, despite adhering well to the Mediterranean diet, the sample exhibited excess body weight and fat mass percentage, as well as reduced muscularity. This may be due to physical inactivity, which is a feature of nearly half of cases.

Anthropometric study of body proportions: a citizen science event during a Leonardo da Vinci celebrative exhibition

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The study of body proportions originates from the aesthetic needs of artistic representations of the human body, dating back to ancient Egyptians, Greeks and Romans times. From the 15th century onward, the main reference for such proportions has been Leonardo da Vinci's drawing known as the Vitruvian Man, which visually represents the descriptions provided by Vitruvius Pollione (c. 80–20 BCE) in De Architectura, and had already been roughly sketched by Francesco di Giorgio Martini a few years before Leonardo. According to this ideal reference, height and arm span should therefore be equal in length for a body to be considered proportionate.

In anthropometric studies, arm span (or wingspan) is generally of limited interest, mostly restricted to areas such as sports as basketball and football, where a wide wingspan can offer advantages in specific phases of the game, contributing to a better performance.

To assess whether the equivalence of these two body measurements is truly common or rare, and to determine which one tends to prevail over the other, a survey was carried out on a sample of the general adult population. The opportunity arose to combine a scientific survey based on anthropometric standards with a Kinect-based 3D body scannering a "playful" setting, as part of a citizen science project conducted in Turin (2019/2020) titled Delle misure universali de' corpi. The event was curated by A. Dino, A. De Bortoli and M. Flores (Unito), scientific project by M. Micheletti Cremasco and A. Giustetto and illustrated by A. Fenoglio (DBIOS UniTo); installation by auroraMeccanica s.c.a r.l. Turin.

Through an interactive exhibit set up at the Galleria Sabauda of the Royal Museums, visitors could compare their own physical proportions with the ideal ones of Leonardo's Vitruvian Man. From the measurements of 2.747 adult subjects (1.435 males and 1.312 females) collected using the Kinect scanner (with 326 subjects also measured using anthropometric instruments, including 173 females and 153 males), it was found that in over 90% of cases, height exceeds wingspan. Only in 6% of female subjects and 10% of male subjects wingspan was greater than height. Virtually no one matched Leonardo's ideal proportions. Comparisons between Kinect measurements and traditional anthropometric ones are reported to help inform future research on the scientific reliability of such applications within citizen science initiatives, as part of the university's third mission activities.

Associations Between Vegetarian Diet and Bioelectrical Impedance Parameters: Insights into Body Composition and Cellular Health in Young Adult Women

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Vegetarian diets are becoming increasingly popular among young adults. The aim of this study is to investigate the impact of such a diet on body composition. Methods: A sample of 202 female young adults, mainly university students from Slovakia, consisting of 73 vegetarians (22.03 ± 2.84 years old) and 129 omnivores (21.26 ± 2.34 years old) was analysed. Body composition was measured using a bioelectrical impedance analyser, the InBody 770. Information on participants' diet was collected using a modified version of the WHO STEPS 2014 questionnaire. Results: Linear regression analysis showed that a vegetarian diet and eating after 18:00 were independent predictors of the body composition parameter reactance (Xc; p = 0.017), while a vegetarian diet also influenced phase angle (PhA; p ¡ 0.001), total body extracellular water ratio (ECW; p = 0.004), left arm ECW ratio (p = 0.004), trunk ECW ratio (p = 0.004) and left leg ECW ratio (p = 0.007) were also significantly affected after adjusting for smoking, allergies, vitamin D and physical activity. Conclusions: Vegetarians had lower values of Xc, PhA, and a higher ECW ratio in all body segments studied, indicating a potentially less favourable state of cellular health and fluid distribution compared to omnivores. These results suggest that a vegetarian diet may have a negative effect on bioelectrical impedance parameters related to body composition in young adult women. Further research is needed to investigate the long-term health effects of these differences.

Sessione

Paleoantropologia

comunicazioni orali

Sandrone cracks a half-smile. Virtual reconstruction and analysis of the Oreopithecus bambolii mandible.

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Oreopithecus bambolii is a fossil primate that lived during the latest Miocene in the regions corresponding to present-day Tuscany and Sardinia. Its remains have been found in a cluster of fossiliferous localities in southern Tuscany and in a single site in northern Sardinia. At that time, these areas were part of a paleo-archipelago made up of several islands, resulting from complex tectonic and sea-level changes in the central Mediterranean region. The most iconic specimen of this species is IGF11778, a nearly complete skeleton commonly referred to as "Sandrone." A major challenge in studying this specimen is its significant diagenetic fragmentation and deformation. In this study, we applied a virtual retrodeformation method to reconstruct the mandible of IGF11778 and to evaluate its morphological affinities with a comparative sample of 300 catarrhine primates. We selected a set of homologous landmarks previously used in studies of mandibular variation among catarrhines, restricting the analysis to 31 landmarks that could be reliably placed on IGF11778. The comparative sample comprised 300 previously published specimens representing the natural range of mandibular variation in extant and fossil catarrhines.

To isolate the deformation component in IGF11778, we used an algorithm that fits a hyperplane to the principal component scores of the reference sample. This hyperplane is assumed to represent biologically plausible, undeformed variation. The deformed specimen was then projected orthogonally onto the hyperplane to obtain an estimated undeformed shape. Based on this configuration, we analysed the morphology of the mandible in relation to that of living non-human catarrhines.

After Procrustes superimposition, a principal component analysis was conducted on the Procrustes coordinates. The first principal component reflects allometric variation among catarrhines. By plotting PC1 against centroid size, we identified two main allometric trajectories: one shared by all great apes, and another shared by the remaining catarrhines and lesser apes (i.e., gibbons). IGF11778 mandible falls within the latter trajectory, suggesting a shared allometric pattern among catarrhines prior to the divergence of Hylobatidae and Hominidae.

Sandrone in the trees: swinging through the past of Oreopithecus bambolii

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Oreopithecus bambolii is a Late Miocene hominoid from the Tosco-Sardinian bioprovince which has prompted discussions about its locomotor adaptations, manipulative capabilities, and phylogenetic positioning for decades. In the present study, a virtual approach was applied to investigate *Oreopithecus* locomotion through the analysis of postcranial skeletal elements of the nearly complete fossil IGF 17788 known as "*Sandrone*" housed in the Geological and Paleontological Museum of Florence, Italy. We analysed the morphological traits of proximal epiphysis of the humerus and carried out a geometric morphometric analysis of the scaphoid, comparing *Oreopithecus* with a large sample of extant and extinct primate genera to minimize the impact of taphonomic deformation. In addition, linear morphometric analyses were performed on cranial and postcranial elements (mandible, humerus, hands, and femur). Despite its relatively large body size, the skeletal proportion of *Oreopithecus* aligns it with lesser apes. The principal component analysis, conducted on the surface areas of the humeral head and the greater and lesser tubercles across 26 primate genera, positioned *O. bambolii* within extant suspensory primates, such as Hylobatidae, Ponginae and Atelinae. The geometric morphometric analysis of the scaphoid was carried out due to its role in inferring locomotor behaviour. Hominoids exhibiting a fused scaphoid–central bone were excluded from the analysis to ensure reliable comparisons. The *Oreopithecus* scaphoid showed large size and morphological similarities to *Pongo* consistent with prior literature. In addition, its trajectory in morphospace aligns it to hylobatids. In conclusion, the integration of linear and geometric morphometric data supports an interpretation of suspensory locomotor adaptation of *O. bambolii* and may suggest a hominoid mainly involved in forelimb-dominated suspension, broadly similar to Asian apes. Future analyses will expand the investigation including additional elements such as vertebrae, metacarpals, and other carpal bones, some of which may also contribute to clarifying the phylogenetic positioning of *O. bambolii*.

Inferring locomotor repertoires of South African australopiths from distal tibial trabecular architecture

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South African hominins display variation in ankle joint morphology, with each taxon showing a different combination of ape-like and human-like features, as well as unique traits. However, the functional implications of these morphological differences on ankle posture and loading remain insufficiently tested through internal bone architecture analysis. This study examines the trabecular structure of five South African hominin distal tibiae within a comparative context of extant hominoids and cercopithecoids to infer their joint loading patterns at the talocrural joint and, in turn, locomotor behavior.

Using microCT data, we investigate the following fossil specimens: StW 358 and StW 389 from Sterkfontein Member 4 (3.4–2 Ma), attributed to *Australopithecus*; StW 567 from Sterkfontein Member 5 East (undated), attributed to Homo sp.; U.W. 88-97 from Malapa (1.98 Ma), attributed to *Australopithecus sediba*; and SWT1/HR-2c from Swartkrans Member 1 (2.3–1.7 Ma), attributed to *P. robustus*. Our extant sample includes distal tibia of *Homo sapiens* (N=15), *Pan troglodytes* (N=23), *Gorilla gorilla* (N=13); *Pongo* sp. (N=7), hylobatids (N=7), and *Macaca fascicularis* (N=5). We used canonical holistic morphometric analysis (cHMA) to holistically compare relative bone volume fraction (rBV/TV) distributions throughout the distal tibia.

A principal component analysis of rBV/TV distribution revealed a clear separation along PC1/PC2 between humans and other taxa. In PC2, great apes separate from Asian hominoids, distinct from macaques. The Sterkfontein M4 *Australopithecus* tibiae exhibit an intermediate pattern with a human-like concentration of high rBV/TV centrally — indicative of a vertically loaded ankle during bipedalism — associated with an ape-like concentration anteriorly, possibly related to loading in ankle dorsiflexion. StW 567 shares this pattern but with higher posterior-central rBV/TV and an observable epiphyseal line. *A. sediba* displays a human-like central concentration, with an Asian hominoid-like concentration of high rBV/TV antero-laterally, possibly related to hyperpronation during bipedal gait. The *P. robustus* specimen from Swartkrans shows a gorilla-like rBV/TV distribution, suggesting frequent dorsiflexed and plantarflexed ankle postures, possibly related to a high degree of vertical climbing in their locomotor behaviour.

These findings support hypotheses of variation in bipedal gait and behavioural repertoires among hominin taxa.

Taung's hidden smile: the morphology of permanent teeth revealed a century later

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The Taung fossil has played a central role in the historical development of paleoanthropology, redirecting the interest of earlier scholars to the African continent as the cradle of humankind. This specimen is the Holotype of the species *Australopithecus africanus* and was first published by Dart in 1925.

In the 100 years following Dart's original publication, numerous works have focused on the erupted deciduous teeth and first permanent molars, associating the Taung specimen with fossils from the sites of Sterkfontein and Makapansgat, and distinguishing it from those from Swartkrans, Kromdraai, and Drimolen. While the pattern of the skull's developing permanent dentition has been investigated radiologically, the morphology of these teeth has not yet been examined in detail using advanced radiographic methods. This work contributes to our knowledge of the permanent dentition of the Taung specimen through the employment of micro computed tomography (CT) scans of the maxilla and mandible to describe and provide metrical information on the crowns of its permanent teeth. The permanent dentition was manually segmented from microCT scans although the high density and heavy mineralization of the bone surrounding the developing teeth, along with the occurrence of calcite encrustations on some of the crowns hindered resolution of some fine-scale morphological details. We here present preliminary descriptions of the outer enamel surfaces of the developing permanent teeth. We also recorded standard MD and/or BL crown measurements from our segmented 3D models of the unerupted dentition using Avizo v. 7.1 software. We compare these linear measurements to samples of *A. africanus* (specimens from Sterkfontein and Makapansgat) and *Paranthropus robustus* (specimens from Kromdraai, Swartkrans, Drimolen, Cooper's. Gondolin, and Sterkfontein Member 5). Overall, the MD and BL diameters of the Taung infant's developing permanent crowns show similarities with the *A. africanus* sample and notable differences from the *P. robustus* dental sample.

Volumetric and Shape Divergences Between Brain and Endocast: Methodological Considerations for Paleoneurology

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The study of brain evolution has long been a significant and debated topic in paleoanthropology. Attempting to analyze and reconstruct soft tissues such as the brain based on proxies is inherently a complex process. In recent years, much research attention has focused on better understanding the relationship between the brain and endocast, as well as the validity of the latter as a proxy. This study presents comparative analyses of the brain and endocast in a sample of 37 living individuals, utilizing a methodological approach that integrates volumetric and geometric morphometric techniques to provide a comprehensive assessment of their correspondence. High-resolution magnetic resonance imaging (MRI) data facilitated the precise segmentation and extraction of soft tissues, defining the brain hull (brain and meningeal layer) for accurate morphological representation. Endocasts were reconstructed from Computed Tomography (CT) scans, capturing the imprint of the brain's outer surface on the inner cranial vault. To evaluate the impact of segmentation parameters, both brain and endocast were segmented using five distinct threshold values. Volumetric assessments were conducted using several software packages to ensure methodological robustness and cross-validation. To conduct the geometric morphometric analyses, a 16-landmark configuration was optimized (following the identification of homologous structures on endocasts and brains) and manually placed. Subsequently, a dense cloud of 1000 semilandmarks was generated to capture finer surface detail.

Data demonstrated a robust positive correlation between overall brain and endocast sizes. However, our analyses revealed significant volumetric variations as a function of the segmentation threshold values. Notably, endocast volumes exhibited a positive correlation with increasing thresholds, whereas brain volumes showed an inverse relationship. Furthermore, Principal Component Analysis (PCA) performed on landmarks and semilandmarks configurations delineated a degree of separation between the distribution of endocast and brain shapes within the multivariate morphospace, indicating inherent morphological disparities that extend beyond simple size differences. Collectively, these observations underscore the complexities and potential limitations inherent in using endocasts as a direct proxy for inferring detailed brain morphology in both extant and extinct species.

Steinheim: the new face of a hominin from the Middle Pleistocene of Germany

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The hominin cranium from Steinheim an der Murr (Baden-Württemberg, Germany) is one of the most important specimens in the European human fossil record. It is an almost complete cranium discovered in 1933 in a gravel pit and roughly dated to OIS 9 (i.e., 300–320 ka to 250 ka) by biochronology. Despite overall good preservation, it lacks a significant part of its left side comprising the facial skeleton and the anteriormost portion of the neurocranium. The cranium SMNS-P-17230, better known as Steinheim, also shows substantial signs of taphonomic deformation, which affects its morphology and has led to different taxonomic attributions in the past. An association with the Neanderthal lineage and possible attribution to *Homo heidelbergensis* became increasingly accepted. The debate surrounding the specimen's taxonomy was due to the peculiar morphology of the preserved portion of its facial skeleton, greatly influenced by multiple taphonomic distortions. Throughout its history, the cranium has been shown without the missing part of the left side. Although fragments of the upper left side of the face were found alongside the cranium, these have been generally overlooked by the scientific community and were only described in a relatively recent German review by Wahl and colleagues (2009). The five larger fragments are labeled T1-T5, while five additional smaller fragments (around 1 cm) have no individual labels and are difficult to refit into the fossil. T1-T5 vary between 2 and 6 cm in maximum length. T1-T3 are possibly part of the left orbit, while T4 seems to be part of the zygomatic arch, and T5 possibly belongs to the inner surface of the neurocranium. Here, we present the digitization by computed tomography of the fragments and an attempt at their integration into a digital reconstruction of the cranium. Both the fragments and the cranium underwent a new micro CT scanning by the Paleoanthropology working group (University of Tübingen), which also allowed a high-quality digital removal of sediment from the virtual model of the fossil. The fragments were then aligned on a corrected model, performed through a published protocol of retrodeformation. The possibility of appreciating an unknown missing portion of the Steinheim face contributes to better assessing possible affinities of this specimen with penecontemporaneous populations of the European Middle Pleistocene.

Beyond Robusticity: Exploring functional and phylogenetic traits in the Neanderthal calcaneus

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While Neanderthals and H. sapiens share several foot features, Neanderthals exhibit unique adaptations in their foot structure, which provide important insights into the evolutionary pressures and locomotor demands specific to their lineage. Neanderthals exhibit distinct calcaneal traits like larger talar facets, a projecting sustentaculum tali, and a broader tuberosity. These differences from modern humans are thought to reflect greater robusticity linked to experiencing higher loads in Neanderthals, but general postcranial robusticity of Neanderthals may not be sufficient to explain their calcaneal uniqueness. This study aims to determine whether Neanderthal external calcaneal shape primarily results from functional behaviors, phylogenetic heritage, or derived features. A total of 85 adult calcanei (7 Neanderthals and 78 H. sapiens) were analyzed using a 3D geometric morphometric approach. The Neanderthal sample includes Regourdou 1, La Chapelle-aux-Saints, La Ferrassie 1 and 2, El Sidrón SD-2192 and SDR-112, and Spy 2. The H. sapiens sample includes Early and Late Upper Pleistocene individuals, Holocene hunter-gatherers, Iron Age individuals, and Post-Industrial groups, representing diverse mobility strategies — from highly mobile hunter-gatherers, either barefoot or wearing soft foot coverings, to highly sedentary post-industrial groups wearing hard footwear. Calcaneal shape variation was assessed using a 3D template of 276 (semi)landmarks. Generalized Procrustes analysis was followed by statistical analyses, e.g., Procrustes ANOVA and PCA, to assess group differences. Neanderthal calcanei exhibit a combination of distinct traits compared to H. sapiens: a more medially convex and laterally expanded posterior talar facet, an enlarged anterior talar facet, a more plantarly oriented sustentaculum tali, and a markedly reduced lateral plantar process of the tuberosity. These traits support the hypothesis that Neanderthals had a pronated foot posture, likely reflecting different force transmission at the heel at touchdown. A short antero-posterior sinus tarsi may be an archaic trait shared with other Pleistocene *Homo* fossils. Shared features with H. sapiens hunter-gatherers, such as a broad calcaneal tuberosity, suggest high load from long-distance barefoot travel on natural substrates. While H. sapiens calcaneal morphology varies with levels of mobility and footwear, Neanderthal calcanei display a mixture of derived, ancestral, and functional traits.

ERC Project “Last Neanderthals” and the fire making skills of Homo neanderthalensis

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Full control of fire is one of the most important behavioral acquisitions of our genus. It expanded the adaptive capability of early humans, triggered many co-evolutionary processes, and enabled some of the cognitive skills unique to our species. To date, several contrasting pieces of evidence fog the picture of how early humans incorporated fire in their life and how they became able to fully control it by starting it at will. In particular, contrasting evidence exists regarding the fire skills of Neanderthals, our closest relatives. There is no doubt that Neanderthals, like archaic modern humans, were using fire extensively, at least in specific periods of the Pleistocene. However, while some evidence suggests that Neanderthals could have struck flint and pyrite to produce sparks, no compelling evidence exists that they were able to start a fire in every environmental condition as we, modern humans, do. The ERC Synergy project “Last Neanderthals”, among other goals, seeks to achieve a deeper understanding of the ability of Neanderthals to start a fire by identifying overlooked fire strikers, looking for pyrite dust and microscopic remains of organic materials used as tinder, and extract fire-derived organic molecules such as Pyrogenic Polycyclic Aromatic Hydrocarbons in the archaeological deposits of over 30 Eurasian archaeological sites dated between 60,000 and 40,000 years ago. The new data collected will invaluably contribute to advance our understanding of the fire-making skills of the last Neanderthals, adding to the research on their adaptability, competitiveness with Homo sapiens and eventually their demise around 40,000 years ago

A multi-stage mortuary landscape in the Late Upper Paleolithic: genomic and bioarchaeological evidence from Arene Candide Cave (Italy)

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With a minimum of 22 individuals interred over a millennium, the Epigravettian “necropolis” of Arene Candide Cave (ca. 12,800–11,800 cal BP) stands as a major source for understanding the biological composition, social structure, and funerary practices of Late Pleistocene hunter-gatherers. Dominated by a 90-meter-high white sand dune rising to its entrance, the site was likely a ritual landmark, used by local groups to reaffirm territorial claims through ancestral ties. A complex, multi-stage mortuary program was enacted within the cave, involving manipulation and secondary deposition of skeletal elements – particularly crania – from previously buried individuals, placed in and around new inhumations. These practices, along with double burials, suggest efforts to establish ritual links between individuals, possibly reflecting kinship or shared congenital conditions.

Indeed, several individuals exhibit skeletal dysplasias, a phenomenon also seen in other European Gravettian and Epigravettian groups. This supports the idea that formal burial was a selective rite – reserved for “exceptional events and exceptional people” – serving to ritually contain biological uniqueness and negative events such as violence or trauma. Still, key questions remain regarding the span of the cave’s funerary use, kinship relationships among the deceased, and the prevalence of congenital conditions – questions that can be addressed through ancient DNA (aDNA) analysis.

In this study, we successfully extracted DNA from 11 individuals following strict aDNA protocols optimized for highly fragmented molecules. Shotgun sequencing and a target enrichment approach were used to reconstruct mitochondrial and nuclear genomes. Initial results identified at least three female individuals, including one adult (AC3) originally sexed as male underscoring the limitations of morphological sex estimation, especially in Paleolithic contexts. This individual was previously diagnosed with hypophosphatemic rickets, a rare X-linked dominant disorder, based on limb bowing and diffused enthesopaties. Further genome data will explore the genetic bases of this condition, mainly linked to mutations of the FGF23 gene.

All mitochondrial DNA haplogroups are closely related and include individuals with identical haplotypes, suggesting a shared maternal lineage. Ongoing genomic analyses will help clarify the presence of a tightly knit kin network among Epigravettian foragers buried at Arene Candide.

Between Normal Variation and Disease: Craniofacial Abnormalities in an Upper Paleolithic Hunter-Gatherer from Maritza Cave (Abruzzo, Italy)

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Upper Paleolithic European hunter-gatherers are well known for their symbolic art, personal ornaments, sophisticated hunting techniques, long-distance exchange networks, and complex burial practices. Their skeletal remains also exhibit a notable frequency of developmental skeletal and dental anomalies. Some of these conditions are extremely rare, of unknown aetiology, or may have a genetic basis—raising important questions concerning inbreeding levels, biological resilience, and social care within these populations.

The skull of the adult male individual from Maritza Cave (Mtz2 – L'Aquila, Abruzzo) shows clear evidence of abnormal bone remodeling, particularly in the craniofacial region (e.g., os zygomatic), with marked bone thickening. Distinctive features include a prominent supraorbital ridge, enlarged mandible, frontal bossing, and hypertrophic mastoid processes. These traits are consistent with pathological or chronic conditions that typically develop after the completion of skeletal growth, such as Paget's disease or acromegaly—both adult-onset disorders characterized by excessive and disorganized bone remodeling, often affecting the craniofacial skeleton.

To better quantify the morphological features of the Mtz2 skull and refine the diagnostic interpretation, virtual anthropology and geometric morphometric (GM) techniques were applied. Landmarks, curve, and surface semilandmarks were recorded on 3D digital models, focusing on the frontal bone, facial skeleton, and temporal bone. After Procrustes superimposition, data were analyzed using Principal Component Analysis (PCA) to explore variations in both shape and form (no size adjusted coordinates) space. Mtz2 was compared with Epigravettian, Mesolithic and Neolithic adults from Italy, two documented cases of pituitary gigantism from infancy, and modern individuals from the Anthropology Museum of the University of Florence. Preliminary GM results showed that, in the form space, the two pathological individuals clearly separate from the comparative sample, with Mtz2 clustering near them, alongside only a few other paleolithic individuals. However, in shape space, no clear pathological signal was observed either in Mtz2 or in one of the two individuals diagnosed with gigantism.

Although the preliminary analysis reveals some morphometric affinities between Mtz2 and the individuals with pituitary gigantism, it does not provide definitive evidence of acromegaly in this case based solely on GM data.

Sessione

Paleoantropologia

poster

On the traces of the Phylum. Neotenic Macroevolution, in light of the explanatory scope of Paleolaringology

Antonia Bertocchi

Context: The report develops the discourse on neotenic macroevolution, proposed by me at Congress AAI 2003 (Bertocchi A.: “Il ruolo della Neotenia umana, nella variabilità genetica e culturale” report presented at the XV Congresso A.A.I. on the theme: “Variabilità umana e storia del popolamento in Italia”-Università di Chieti, 28-30 September 2003 ACTS published by Edigrafital, Teramo. 2005).
Object The topics covered are based on the neotenic descent of the larynx. An event that “does not in itself entail any advantage, but rather, the risk of suffocation, and that allows to overturn the hypothesis that the development of the brain has allowed the man to speak. On the contrary, it would have been precisely the possibility of refined sound production, given to us by the neotenic lowering of the larynx, to shift the selective push in favor of those who possess a brain capable of understanding and building language”.
This approach results in a methodology which allows:
1)-the solution to the problem of language ability in Neanderthals. 2) consideration of the role of the dimensions of the pelvis and the birth canal, as contexts of localization, temporal range and geographical area in which the initial moments of the ancestral neotenic scene took place.
3-) Two scenarios of the so-called “Cradle of Humanity” are compared: one in South West Africa (Svarktrans) and one in the Rift Valley (in a probable area isolated by tectonic phenomena. in which two species: one of the genus Paranthropus and one of the genus Homo have been found to coexist.
Objective ‘ The weaving of a “Neotenic hypothesis of human evolution” for which writing and verification, I invite colleagues to a collective and transdisciplinary work.
I hope that this contribution will arouse interest in the academic institution of Paleolaryngology (a discipline still neglected) as it seems to be able to provide the innovative methodology, whose high explanatory scope, allows innovative excursions in the field of paleoanthropological research, here open to reflection and discussion.

The derived facial morphology of Neanderthals in the light of cranial and cervical constraints

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A critical review of the scientific literature show how masticatory and respiratory constraints may have contributed to the Neanderthals' distinctive facial architecture. Clinical studies on modern humans demonstrate that maximal mouth opening involves not only a downward and backward rotation of the mandible, but also cranial retro-extension and accentuation of the cervical lordosis. This compensates for the posterior movement of the mandible, optimizes the alignment of the upper airway, and reduces the risk of compression on the neurovascular structures of the retromandibular region. In Neanderthals, by contrast, several studies indicate a reduction in the degree of cervical lordosis and a more limited range of flexion-extension in the cervical spine (as evidenced by Coletti et al., 2025). This reduced cervical mobility may have constrained the extent to which cranial retro-extension could assist in maximal mouth opening. Consequently, the mandible appears to have undergone compensatory adaptations: more elongated mandibular body, presence of a pronounced retromolar space, and reduced gonial angle collectively enhance gape efficiency while minimizing reliance on cervical extension. This preserves temporo-mandibular joint function and suggest an integrated adaptation of the masticatory system to a more rigid neck. At the same time, respiratory constraints likely played a significant role. The presence of a large nasal cavity, increasing both the internal volume and surface area, contributed to the overall forward projection and enlargement of the midface. Thus, the combined influence of masticatory, respiratory, and postural demands provides a comprehensive functional framework for understanding the distinctive anatomy of the Neanderthals.

Coletti B., A. Papini, M. Boggioni, F. Di Vincenzo and G Manzi (2025). Reduced neck mobility in Neanderthals: anatomical constraints and biomechanical implications. 26° Congress A.A.I., Cagliari.

Reduced neck mobility in Neanderthals: anatomical constraints and biomechanical implications

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The Neanderthal cervical spine displays a specialized morphology that prioritizes stability over mobility. This study presents a critical review and synthesis of the scientific literature concerning Neanderthal cervical morphology. Our analysis aims to provide a comprehensive and integrated perspective on the evolutionary trajectory of this taxon (compare Boggioni et al., 2025), with particular emphasis on the distinctive features of the cervical spine. Compared to modern humans, Neanderthal cervical vertebrae exhibit distinct features: a shorter cervical column, longer and more horizontally oriented spinous processes, broader articular facets, and significantly reduced cervical lordosis. These traits indicate increased neck stability, likely to support robust cranial and upper limb mechanics within a tightly integrated pectoral girdle system. In addition, Neanderthal inner ear morphology provides complementary evidence: smaller anterior and posterior semicircular canals, associated with reduced sensitivity to flexion-extension and lateral bending, contrast with a relatively enlarged lateral canal, linked to enhanced rotational detection. Other anatomical features – such as absence of the tubercle for the transverse ligament of the atlas, reduced mastoid process and modified ligamentum nuchae morphology – further align with a functional complex optimized for head and neck stabilization under high mechanical loads. Taken as a whole, this combination of features highlights a cervical spine tailored for stability within a broader morpho-functional framework, distinct from the more flexible cervical architecture of Homo sapiens.

Boggioni M., A. Papini, F. Di Vincenzo, B. Coletti, and G Manzi (2025). The derived facial morphology of Neanderthals in the light of cranial and cervical constraints. 26° Congresso della A.A.I., Cagliari.

Early LSA Homo sapiens lithic technology in Eastern Africa: Insights from the Naisiusiu Beds type section, Oldupai Gorge, Tanzania.

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This study investigates the behavioral aspects of lithic tools production by Late Stone Age (LSA) Homo sapiens at the Naisiusiu Beds type section in Oldupai (Olduvai) Gorge, Tanzania. By integrating historical assemblages with newly acquired data, the research re-evaluates the site's significance within the broader LSA framework and explores human behavioral variability during the Late Pleistocene.

We combined data from lithic collections gathered by the Leakeys in 1931 and 1969 with new materials recovered during a 2024 surface survey by the THOR (Tanzania Human Origins Research) team.

Our technological analysis included the reconstruction of reduction sequences and the systematic recording of discrete and metric attributes on both cores and products. In addition, a 2D Geometric Morphometric (2DGM) approach was applied to a subset of 105 complete blanks and retouched tools to investigate patterns of shape variability. Comparative PCA was also performed to assess potential regional affinities.

The results provide new insights, particularly regarding patterns of lithic volume exploitation, highlighting a clear preference for narrow-sided and wide-faced volumetric cores used for the concurrent production of blades and bladelets. A limited, but still evident use of the bipolar technique was also observed, almost exclusively on wide-faced cores. 2DGM analysis revealed significant shape variation related to blank type and retouch intensity, supporting hypotheses of consistent hafting orientations. Furthermore, the backed microliths' metrics from the Naisiusiu Beds closely align with those from Enkapune Ya Muto (Kenya) in the PCA space, suggesting broader regional connections within LSA technological traditions and pointing toward a distinctive Central Rift Valley trajectory.

In conclusion, this study highlights the technological dimensions of the LSA occupation at Oldupai in the Late Pleistocene. The modern reanalysis of historical lithic collections demonstrates the value of integrating historical and new data, offering a renewed perspective on the technological behavior of LSA population at Oldupai, shedding light on Homo sapiens adaptations in Eastern Africa during the Late Pleistocene.

Taxonomic assessment of a human deciduous molar found in the Lower Palaeolithic layer from the Observatoire Cave (Principality of Monaco)

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The Liguro-Provençal corridor, spanning southeastern France and western Italy, shows continuous human occupation from the Lower to Upper Palaeolithic, testified by lithic, faunal and human fossil finds. Among its sites, Observatoire Cave, in Monaco's Exotic Garden, holds the earliest archaeological evidence of human presence in the Principality. First excavated (1916-1920) by Léonce de Villeneuve under Prince Albert I, it was then investigated by Louis Barral and Suzanne Simone in the 1950s and 1980s, alongside restoration and musealization. Since 2016, Olivier Notter and Elena Rossoni-Notter have resumed excavating the lowest levels. The site's stratigraphy comprises three techno-cultural macro-complexes: an upper complex (Proto-Aurignacian, Aurignacian, Gravettian), a middle complex (Mousterian), and a lower complex (Acheulean-Clactonian, Pre-Mousterian). In 2020, when excavating the lowest levels, a human tooth (Obs1) was found in sieved sediments coming from the site's surface and wall. To date, this is the only human fossil from a Palaeolithic context in Monaco, potentially dating back to the Lower Palaeolithic, due to its context of recovery. Here, we share a morphological description and 2D geometric morphometric (GM) analysis, aimed at clarifying its taxonomic attribution, aided by a pathological and taphonomic study. A 2D GM analysis of the crown outline was performed. A genetic analysis of Obs1 was also conducted. Obs1 is an upper right second deciduous molar, with four main cusps and a pronounced Carabelli's trait. A deep tooth-pick groove filled with concretion is visible on the distal cervical margin. GM results show that Obs1 differs from Neanderthals and early *H. sapiens*, but aligns with Upper Palaeolithic and recent *H. sapiens.* Obs1's DNA revealed contamination with exogenous human genetic material. Microscopic analysis of the tooth-pick groove suggests intentional cleaning or pain-relief behaviour, consistent with known Middle and Upper Palaeolithic cases. Our results support its attribution to *H. sapiens*, likely an Upper Palaeolithic child, as no later occupation of the site is known. Evidence suggests its accidental displacement from an Upper Palaeolithic layer into an older one during earlier restoration works. Thus, we could reject the initial hypothesis of an earlier hominin, as assumed by stratigraphy. Obs1 is the oldest human fossil found to date in Monaco, likely from the Proto-Aurignacian, Aurignacian, or Gravettian period.

Sessione

Primatologia

comunicazioni orali

Social Familiarity Enhances Positive Responses to Tickling in Bonobo Infants

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In humans, intense tickling elicits a complex behavioural response characterized by withdrawal, protective movements over sensitive areas, wriggling, and uncontrollable laughter. This reaction is socially modulated: the experience of tickling can be perceived as either pleasurable or aversive depending on the nature of the relationship between the individuals involved. While similar tickling behaviours have been documented in other great ape species, they remain largely understudied. This study investigates the behavioural and vocal responses of bonobo infants to tickling and examines whether these responses vary according to the degree of familiarity with the tickler. Using an experimental paradigm, we collected acoustic and video data from seven bonobo infants housed at the Lola ya Bonobo sanctuary in Kinshasa, Democratic Republic of Congo.

Our findings show that tickling (experimental condition), but not simple touching (control condition), evoked a complex response in the bonobo infants. Furthermore, the infants laughed more, engaged more often in mutual gaze, and more frequently solicited additional tickling when the interaction came from a familiar individual compared to an unfamiliar one. These results suggest that bonobos' responses to tickling are modulated by social bonds, pointing to the possible involvement of empathy-related mechanisms.

Empathy in the Homininae: insights from paly ontagion in gorillas, chimpazees, bonobos and humans

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According to perception–action and neurocognitive models of empathy, behavioural contagion—where a behaviour performed by one individual (the trigger) acts as a releasing stimulus and automatically induces the same behaviour in another (the responder)—can mediate emotional contagion, a basic form of empathy. This transfer is enabled by shared neural representations built between trigger and responder. In this study, we investigated the presence and modulators of play contagion in the subfamily Homininae. From December 2021 to March 2025, we used audio–video recordings and all-occurrence sampling to collect data on play, affiliation (grooming, contact sitting), and agonistic interactions in lowland gorillas (*Gorilla gorilla*; n = 21), chimpanzees (*Pan troglodytes*; n = 16), and bonobos (*Pan paniscus*; n = 35)—two groups per ape species—at Beauval, Palmyre, and Vallée des Singes (France), and Ouwehands Zoo (Netherlands). We used the same method to collect data on human toddlers (*Homo sapiens*, two nursery classes, n = 28) at Melis nursery (Turin, Italy). Data were analysed using GLMMs. We found that play was contagious in all species, as individuals were more likely to start playing after seeing others play (Post-Play condition) than when they had not observed any prior play session in the same socio-environmental context (Matched-control condition). Play contagion level was not species-dependent, but modulated by group demographics (sex and age ratio) and social dynamics (affiliation frequency). Play contagion increased with more males, more young individuals, and higher affiliative rates—consistent with the idea that play, and the related emotion transfer, mostly affects juveniles and males who establish playful networks to build social bonds or compete non-aggressively. These findings—obtained using the same ethological methodology across species—demonstrate for the first time that play contagion is widespread among Homininae and point toward basic empathy being hard-wired shared biology of humans and social great apes.

From Conflict to Comfort: Post-Aggression Affiliation in Toddlers Reflects Non-Human Primate Strategies

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In both human and non-human primates, conflict of interests over resources naturally arises between individuals and can be resolved through tolerance, avoidance, or aggression. Aggression is a social behavior that can negatively affect group cohesion and individual emotional states. However, primates - like many other social species - have developed post-conflict behavioral strategies aimed at mitigating the negative consequences of aggression through affiliation. These strategies include reconciliation between former opponents and triadic contacts initiated by uninvolved third parties (unsolicited) or by aggressors or victims (solicited). In this study, we collected live and video data on post-conflict and anxiety-related behaviors in toddlers (N = 18; 18-30 months old) at the kindergarten "Armando Melis" (Turin, Italy) during their routine daily activities from February to May 2022. To evaluate the presence of such behaviors, we employed the Post-Conflict/Matched-Control method commonly used in similar studies on other social species, particularly non-human primates. We demonstrated for the first time in this age cohort the presence of reconciliation (mean Corrected Conciliatory Tendency 33.12% ± 7.33% SE) and triadic affiliations, both unsolicited offered by third parties toward victims (mean Triadic Contact Tendency-TCT 44.87% ± 12.00% SE) and solicited initiated by the victim (mean TCT 39.24% ± 11.02% SE) or the aggressor (mean TCT 33.76% ± 11.74% SE). Focusing on unsolicited triadic contacts - previously demonstrated in only a few other species - we found a significant decrease in anxiety-related behaviors in victims after affiliation, but neither in aggressors nor in third parties. This finding confirms the presence of genuine consolation behavior. In sum, from as early as 18 months of age, toddlers - like chimpanzees, bonobos, and some macaque species - may possess the capacity to perceive and respond to the emotional states of others. Detecting a peer's distress can elicit emotional arousal in third parties and promote other-oriented behaviors through emotional resonance. As a result, the third party's behavior may be implicitly aimed at reducing the emotional disparity between themselves and the distressed individual.

Automated detection of two lemur species for passive acoustic monitoring

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Passive acoustic monitoring (PAM) is a broadly employed practice in wildlife research, facilitating the non-invasive collection of information on presence, abundance, distribution, and behaviour of vocal species over large temporal and spatial scales. On the downside, the resultant data volume poses significant computational challenges, potentially hampering the data processing and analysis. Lemurs, with one-third of species classified as critically endangered by the IUCN, need meticulous and timely monitoring of wild populations. Many species live in remote forest remnants and possess typical vocal behaviour. Hence, they are optimal subjects for studies relying on passive acoustics. We focused on the vocal activity of indris (Indri indri) and black-and-white ruffed lemurs (Varecia variegata) recorded in Maromizaha Forest (Madagascar) from 2019 to 2023 via passive acoustics. We first built a convolutional neural network (CNN) trained to identify recordings with the presence or absence of indris' songs, loud distinctive vocal sequences, across 66,443 10-minute recordings. Once proved that our network was efficient in detecting songs (accuracy ¿90%; recall ¿80%) and hence that an automatic detection was indeed feasible thanks to a tailored process, we applied the machine learning software BirdNET—a CNN originally developed for ornithological applications—to the automatic recognition of the loud calls of indris and black-and-white ruffed lemurs. We manually adjusted CNN settings and employed the optimal model (achieving precision and recall rates of 90% for both species) to analyse 55,515 10-minute recordings. Lastly, we conducted temporal analyses to examine the species' diel and seasonal vocal pattern. First, thanks to our approach we were able to show that software like BirdNET can indeed be customed to analyse diverse sets of data. This would render the automatic processing of acoustic data viable also with a reduced computational background, increasing the reach of these studies across taxa and locations. Secondly, our study demonstrates the essential role of PAM in enhancing our understanding of the vocal behaviour of these threatened species, crucial for their detection and to provide critical information to plan data collection and conservation efforts and strategies.

Comparative Genomic hybridization (CGH) and satellite DNA pattern in Gibbons to Identify Hybrids

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Gibbons and Siamang include 20 species originated vary recently, characterized, among primates, by a very high level of rearrangements and where speciation is considerably faster than that observed in many other groups of mammals.

The small apes with four genera Hoolock, Symphalangus, Hylobates and Nomascus are included into conservation projects because in endangered status for the loss of their habitat and even because hybrids are common in nature and captivity. Here it is proposed an easy and fast method, the Comparative Genomic Hybridization (CGH) and DAPI inverted banding to detect balanced or unbalanced heterochromatin DNA distribution to study repetitive sequences origin and to identify hybrids. CGH has been performed intra and inter species on four gibbons' samples. The animals' samples coming from different zoo are a Symphylangus syndactilus (2n=50), Hylobates lar (2n=44), and two Nomascus samples (2n=50), one identified from zookeepers as N. leucogenis and the other as N. concolor. The CGH applied intra and inter species permitted to detect repetitive sequences pattern in genomes with telomeric, interstitial and centromeric distribution. Results permit to discuss the origin of these sequences among small apes considering the fact each genus have a representative pattern, with a few repetitive sequences being in common between genera and mostly being genus specific. Through this genus specific distribution of heterochromatin is possible also to identify hybrid due the fact they have two different patterns of heterochromatin in their genomes. Indeed, hybrid present half set of chromosomes with a CGH pattern from each parent. Through this approach and the Dapi inverted (like G bans) pattern analysis was possible to identify the two Nomascus samples as hybrids. The first hybrid having in heterozygosis the CGH pattern of Hylobates lar and Nomascus concolor and the second one of Nomascus leucogenis and N. siki.

This study shows the CGH usefulness in identify genera specific pattern of heterochromatin, hybrid among species and pure lines. For those reasons CGH is worthy to be included in project of conservation purpose.

Sessione
La vita negli ambienti estremi

Comunicazioni

Riding the tide: where land meets sea and risk meets opportunity

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What makes intertidal zones a truly exceptional adaptive challenge is the dramatic variability of their chemical, physical, and biotic parameters, across both spatial and temporal dimensions and at a wide range of scales. These environments represent dynamic land-sea ecotones, shaped by the oscillations of the sea surface caused by gravitational forces exerted by the moon and the sun on the ocean's water mass. The already complex astronomical origins of tidal phenomena are further influenced by the configuration of ocean basins and the local geometry of coastlines. The result is a series of oscillations at the land-sea interface, characterized by multiple harmonic components that can reach levels of local complexity nearly beyond the predictive capacity of even sophisticated computational systems.

The animal organisms inhabiting intertidal systems develop intricate morphological and physiological adaptations—from the molecular to the macroanatomical level—that enable them to achieve significant fitness in such conditions. These are almost invariably complemented by complex behavioral strategies that allow organisms to cope with environmental fluctuations and exploit the unique resources that arise from them.

Thus, spatio-temporal complexity often translates into opportunity, both at the species and community level, with high degrees of species packing. Over the long term, this generates extraordinary evolutionary potential. In this sense, tidal systems have played a pivotal role in biological evolution—from the origin of life itself to the colonization of terrestrial environments, and even the return of terrestrial organisms to the sea. Human demographic expansion, particularly that of *Homo sapiens*, has likewise been closely tied to the capacity to exploit the resources of tidal environments while avoiding their associated risks.

Characterization of free-living nematode assemblages in Mediterranean ports: implications for benthic monitoring

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Ports are maritime zones of high economic importance, hosting a range of activities such as industrial operations, container terminals, petrochemical handling, and tourism. These activities make port areas both sources and recipients of environmental stressors that can significantly impact marine fauna. Effective management of port environments is therefore essential for marine conservation, with environmental assessment and monitoring representing key steps—particularly through the use of bioindicators such as free-living benthic nematodes.

However, a major limitation remains the lack of baseline data for this phylum. To address this gap, we characterized free-living nematode assemblages in seven Mediterranean ports, spanning from the Adriatic to the Ligurian Sea. We analyzed their abundance, taxonomic composition, diversity, and functional traits.

Our results suggest that nematodes may exhibit adaptation to long-term contamination, likely in synergy with other benthic components such as diatoms and prokaryotes. Canonical analysis of principal coordinates allowed us to distinguish faunal assemblages across port basins, while routine distance-based linear modeling identified key environmental drivers shaping nematode communities, including sediment mud/silt content, water depth, and polycyclic aromatic hydrocarbon (PAH) concentrations. These findings highlight the importance of site-specific environmental conditions in structuring nematode assemblages, which limits the identification of a universal set of stress-indicator genera. At present, synthetic indices such as the Maturity Index and Shannon diversity remain the most effective tools for assessing ecological quality in marine ecosystems using nematodes.

Unraveling the impact of urbanization on pollinator insects through a metabolomic approach

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Pollinators play a crucial role in maintaining ecosystem functionality and food security. However, rapid urban expansion is altering their habitats, phenology, and behaviour, favouring species more adaptable to urban settings. In this study, we investigated the effects of urbanization on wild pollinators widely distributed in Italian cities. Using targeted and untargeted metabolomic approaches, we aimed to assess the impact of urban stressors on pollinator populations and explore potential adaptive responses to urban environments, leveraging the capacity of metabolomics to detect subtle physiological and biochemical changes that serve as early indicators of stress and reveal potential metabolic adaptations to urban conditions. In the initial phase, we collected 82 of *B. terrestris* individuals from 12 semi-natural and urban sites in the Milan metropolitan area. Metabolites were extracted from the entire body and first analysed using a targeted approach to quantify malondialdehyde (MDA), a biomarker of oxidative damage. A subset of 30 samples was then analysed through untargeted metabolomics (LC-MS) to investigate broader metabolic responses. Afterwards we expanded the study to a national scale, sampling across four major Italian cities: Milan, Rome, Turin, and Florence, and also including samples of *B. pascuorum* and *Osmia cornuta*. Around 120 *B. terrestris*, 120 *B. pascuorum*, and 60 *O. cornuta* were collected from 28 sites along an urbanization gradient. As before, whole-body metabolites were analysed using LC-MS untargeted metabolomics to assess metabolic changes across varying land use scenarios. Results from the first experimental phase on *B. terrestris* showed clear differences between urban and semi-natural individuals. MDA levels were higher in urban habitats, suggesting greater oxidative stress. We also found consistent changes in stress-related and adaptive metabolites: for instance, urban bumblebees had higher levels of unsaturated phospholipids, possibly reflecting adaptation to higher urban temperatures. Results from the nationwide sampling revealed significant differences in the metabolic profiles of all species along the urbanization gradient. Ongoing analyses will identify key compounds varying with urban intensity. These findings will advance our understanding of how urbanization affects pollinators and inform conservation and urban planning strategies.

Under anthropogenic pressure: honeybees may amplify the effects of retreating glaciers on wild bee diversity and bee-plant networks

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Human impacts on the planet span several scales, from local management activities to global change. Wild bees (Hymenoptera: Apoidea) are important pollinators whose diversity is increasingly undermined by anthropic pressures. While the effects of managed honeybees on wild bee diversity and foraging patterns have been largely demonstrated, studies have rarely been conducted in extreme and fragile environments such as high mountain plains affected by glacier retreat. Here, wild bee biodiversity and bee-plant networks were studied across a 170-year gradient of glacier retreat in Valais, Switzerland. This provides a unique case study, linking global change effects (glacier retreat) and local management activities (beekeeping) with wild bee communities, whose diversity and the robustness of bee-plant networks should be maximised at intermediate stages of glacier retreat and with lower honeybee abundance. A total of 231 wild bees and 105 honeybees were sampled at four stages of glacier retreat, and the plant they were feeding on was recorded. Wild bee abundance and Hill's numbers were strongly influenced by both glacier retreat and the proportion of honeybees over the total number of bees sampled per stage. The most recent areas of glacier retreat were characterised by richer communities (*N, q0*), while *N, q0*, and *q1* were negatively correlated with increasing honeybee proportion. *H2'* (network-level specialisation), bee niche overlap, and connectance increased, and modularity decreased, with increasing honeybee proportion. This suggests that honeybees may monopolise floral resources in poorer environments (i.e. next to and furthest from the glacier front), whereas in habitats with lower proportions of honeybees resources are more evenly distributed among bee species. Glacial retreat stage had weaker effects on network metrics, although older stages were characterised by lower modularity and higher niche overlap. These results have strong conservation implications, since glacier loss is likely to reduce wild bee abundance and support poorer bee communities as succession progresses towards more closed (i.e. forest) habitats. Furthermore, glacier retreat may be changing the floral resources available to wild bees, with honeybees possibly displacing them through competition. Hence, conserving wild bee communities in such delicate environments would benefit from maintaining beekeeping at low levels, especially at most recent and oldest glacier retreat stages.

Beneath Borders: tracing hidden waters and subterranean life in the Reka-Timavo system through environmental DNA

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The Reka-Timavo hydrological system, with an underground course extending approximately 90 km, represents a still partially unexplored environment in the transboundary area between Italy and Slovenia. The environmental DNA (eDNA) approach has significantly enhanced the ability to collect data on species distribution across a wide range of ecosystems.

In this study, we investigated the metazoan community present in the waters of the Reka-Timavo system through eDNA analysis. Sampling was conducted at two sites within the Škocjan Caves Park (Slovenia) and one site in the Luftloch Cave (Italy) between late July and the first week of August 2024. Environmental DNA was extracted, amplified and purified using commercial kits, and sequenced with Illumina technology (NovaSeq6000 SP) at a depth of 2×250 bp.

The three analysed sites displayed distinct biological assemblages. In the Slovenian locations, most of the zooplanktonic community belonged to the phylum Rotifera, particularly the genus Ploima. In contrast, the dominant taxa in the Italian site (Luftloch Cave) were from the phylum Arthropoda, primarily the class Copepoda (*Acanthocyclops spp.*) and class Insecta (order Diptera). Hydrozoan detections in subterranean environments are rare; thus, we highlight findings related to this taxonomic group. DNA from *Craspedacusta sowerbii*, a freshwater jellyfish native to East Asia, was identified in all sampled caves. Although considered common in Western European freshwater systems, this species is often underreported due to its inconspicuous polyp stage. Its detection in the Reka-Timavo system supports a recent report by the Natural History Museum, confirming its presence as an alien species in the upper Timavo (Slovenia).

Additionally, DNA from *Hydra vulgaris*, a species already known in the region, was found in the Slovenian samples. Of particular interest was the detection of *Velkovrhia enigmatica* (Matjašič & Sket, 1971), the only known stygobiotic freshwater hydrozoan, endemic to the Dinaric Karst. This finding is especially noteworthy and warrants further investigation due to its ecological significance.

These preliminary results lay a solid and promising foundation for the application of eDNA methodologies in the study of subterranean environments. The data collected so far suggest that this approach could become a powerful and innovative tool for biodiversity assessment in underground ecosystems, paving the way for more detailed and targeted future research.

Light pollution, global warming, and behavior: an overview from fish models

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The impact of anthropogenic action on aquatic and terrestrial ecosystem is increasingly recognized as the major driver of ecological disruption, altering both biotic and abiotic environmental conditions. In particular, the global warming is accelarating and will likely continue to increase in the coming decades, leading to biodiversity and ecosystems services loss. Although less considered as a stressor, light pollution has become a serious threat by altering physiology and behaviour of both vertebrates and invertebrates. Understanding how the cumulative impacts of global warming and light pollution alter animal behavior is essential for developing effective conservation strategies for natural populations. To date, we have performed a series of experiments in which larvae and adult zebrafish (*Danio rerio*) were exposed in mesocosms simulating these anthropogenic stressors (i.e., heat stress and Artificial light at night-ALAN) to investigate their effects on behavior at different developmental stages. Exposure to ALAN altered total activity, brain lateralization and learning in zebrafish larvae, while more complex cognitive functions were affected in adults. ALAN-exposed adult zebrafish also showed a disrupted daily pattern of behavioral activity, losing their photic entrainment. Moreover, brain transcriptome analysis revealed significant alterations in the expression of core clock (*per1* and *cry1*) and clock-related genes (*aanat2*), and genes associated with immunity, stress response, responses to sensory stimuli, energy production and reproductive processes. When experiencing heat stress during development, zebrafish larvae showed reduced total activity and impaired cognitive performance. Alterations on swimming activity and lateralization were also observed in adult zebrafish exposed to simulated heatwaves. Overall, our findings confirm that global warming and light pollution significantly affects several aspects of zebrafish behavior and cognition at different life stages, with potential consequences for individual fitness. By taking advantages from these results, we have extended our approach to the Mediterranean killifish *Aphanius fasciatus*, a good target species for studying the ecological and evolutionary effects of anthropogenic stressors on Mediterranean coastal ecosystems.

Unveiling the resilience of the blackmouth catshark *Galeus melastomus*: growth, maturity, and habitat use in the Western Mediterranean Sea

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Deep-sea elasmobranchs are particularly susceptible to overexploitation due to their slow growth, delayed maturity, and low reproductive output. Among them, the blackmouth catshark (*Galeus melastomus*) is one of the most frequently captured species in the Mediterranean Sea, acting as a generalist and opportunistic predator across a wide bathymetric range (˜300–1873 m). Despite its broad distribution and significant presence as bycatch in deep-water trawl fisheries, critical aspects of its life history, particularly growth, remain insufficiently understood. Given their relevance for fisheries management, this study aims to advance current knowledge on the species' reproductive cycle, growth dynamics, and bathymetric segregation.

Specimens were collected between 2012 and 2023 from both fishery-dependent and independent surveys in FAO Geographic Subareas (GSAs) 9 (Ligurian and Northern Tyrrhenian Seas) and 11 (Sardinia). Individuals were measured and macroscopically assigned to sex and maturity stages. A subset of 1,201 individuals (788 from GSA9, 413 from GSA11) ranging from 6.5 to 53.0 cm in total length was selected for age estimation via sectioned and crystal violet-stained vertebral centra. Age–length data were analysed through a multi-model inference analysis.

Results indicate a continuous reproductive cycle throughout the year, with a seasonal peak in winter. Females reached sexual maturity at slightly larger sizes than males in both areas (L50: 41.3 vs. 39.2 cm in GSA9; 44.4 vs. 41.2 cm in GSA11). Among the tested growth models, the von Bertalanffy function provided the best fit, as indicated by Akaike's Information Criterion. No significant differences in growth patterns between sexes were observed. Estimated growth parameters for combined sexes were L=60.76 cm, k=0.20, t =–1.30 in GSA9, and L=52.34 cm, k=0.26, t=–0.94 in GSA11. These values were consistent with parameters derived from length–frequency distribution analyses on the whole dataset, thus corroborating the direct ageing protocol.

A marked bathymetric segregation was observed, with immature individuals nearly absent below 1000 m and a female-biased sex ratio beyond 1200 m. These deeper zones may serve as partial refugia from fishing pressure. Together with an extended reproductive cycle and relatively rapid growth compared to other elasmobranchs, these traits may help explain the continued abundance of *G. melastomus* in the Mediterranean despite ongoing exploitation.

Trace elements accumulation and parasitic charge in 4 species of deep-sea chondrichthyes from the Sicilian Channel: results from DEEP-MED project

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Trace element contamination in deep-sea ecosystems is a topic of growing scientific interest, as these environments, although remote, are not free from direct and indirect anthropogenic pressures. In this context, the present study aims to evaluate the bioaccumulation levels in four deep-sea benthic and benthopelagic fish species: *Raja clavata*, *Scyliorhinus canicula*, *Galeus melastomus* and *Chimaera monstrosa*, with the aim of exploring any species-specific differences attributable to ecological and trophic characteristics and correlating the results obtained with the structure and composition of parasitic helminth communities. The concentrations of 17 elements (Al, As, Cd, Co, Cu, Mn, Mo, Ni, Zn, Se, Sr, V, Pb, Cr, Fe, Bi and U) were determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer ElanDRCe). The results allowed to elaborate specific abundance profiles for each species, where arsenic was the dominant element in all the examined taxa, followed, in variable order, by zinc, iron and aluminum. Higher mean bioaccumulation values emerged in *R. clavata* and *S. canicula* compared to *G. melastomus* and *C. monstrosa.* This pattern seems consistent with the different ecological strategies: the first two species show a greater affinity for the substrate and demersal sediments, which can act as reservoirs for trace elements, compared to the other two species more frequently associated with the water column and characterized by greater vertical mobility.

As regards parasites, the species *C. monstrosa* was found to be parasitized only by *Chimaericola leptogaster*, *R. clavata* with 10 species of parasites and a prevalence of 48.15% was the species with the highest parasitological diversity; *G. melastomus* and *S. canicula* showed a very high prevalence that was equal to 98% for both species, with an average intensity between 46.3 and 48.3 but with communities represented by three and four species of parasites: among these, *Grillotia sp.* was the one with the highest intensity, with peaks of over 300 individuals per host, located almost exclusively in the muscle tissue. The work contributes to improving the understanding of bioaccumulation processes and their relationships with parasite loads in deep-sea fish and lays the basis for future ecotoxicological and food risk assessments in bathyal contexts of the Central Mediterranean.

New data on the inter- and intra-population variability of the eco-morphology and trophic ecology of the longspine snipefish, *Macroramphosus scolopax*, linnaeus, 1758

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Despite the great ecological and social relevance of deep-sea ecosystems, and the growing anthropogenic impacts affecting them, the knowledge base on the biodiversity and trophic dynamics of deep-sea communities remains relatively poor. In this context, it is important to provide valuable data on both the eco-morphology of deep fauna and the ecological dynamics regulating deep-sea communities. In the Mediterranean Sea, the long spine snipefish, *Macroramphosus scolopax*, is one of the most widely distributed and abundant teleost species, ranging between 25 and 600 m of depth. It is a fast-growing short-living cosmopolitan species, essential in the energy transferring between different depth strata and domains. Present research aims to examine the intra and inter population variability in feeding habits and eco-morphology of two *M. scolopax* populations inhabiting the Italian waters.

A total of 533 specimens were collected from trawl fishery operating in the central-western Mediterranean Sea around Sardinia (220 Ind.), and in the southern and central Tyrrhenian Sea (313 Ind.). Once transported in laboratory, they were measured, weighted, and divided in two size classes (Class I $\leq$ 110 mm; $ClassII$>110 mm). Once sampled otoliths and stomachs, sagittae were analysed for age estimation and photographed for morphometrical and shape analyses, while stomachs contents were examined to reconstruct the feeding habits. The prey-specific index of relative importance was calculated for each prey, also assessing, in both populations, the niche breadth, trophic niche overlap and trophic level of each identified age and size class.

Results confirmed the capability of the species to feed on both planktonic and benthic organisms in the two studied areas, partitioning the trophic resources, at intra-population level, between the different size and age classes. Smaller and younger individuals of both populations showed more pronounced planktivorous habits than the larger and older ones, with otoliths morphology and shape reflecting the benthopelagic habits of the species in both the studied areas. At inter-population level, the *M. scolopax* specimens showed slightly differences in otoliths' features and diet composition. This highlighted the capability of the species to adapt to the variability in resources availability and environmental features between different geographical areas, an essential features to thrive in the deep oligotrophic Mediterranean ecosystems.

Reproduction in deep sea cephalopods: adaptations and mating errors in ***Rossia macrosoma*** (Delle Chiaje, 1830)

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Cephalopods are an important component of marine ecosystems around the world, and most species (coleoids) are considered semelparous, short-lived and fast-growing. Reproduction in coleoids has long been studied in coastal species and schematised as a internal fertilisation of small and numerosus eggs within a single reproductive event followed by death. More recent studies on some deep-sea cephalopods have revealed how they have a great flexibility in adapting to the environment, showing, peculiarities in morphological and/or ethological reproductive aspects. This is in response to some limitations such as difficulties in meeting the mate due to the absence of light.

To upgrade this issue on the stout bobtail squid *Rossia macrosoma*, 88 males and 156 females at different maturity stages were sampled by trawl net in Sardinian waters (western Mediterranean Sea) at an average depth of 500 m. *R. macrosoma* doesn't exceeding 84 mm (females) and 60 mm (males) in mantle length and reaches the mature stage starting from about 30 mm. Both sexes produce a relatively low number of sexual products with large dimensions. In females, mature ovaries contained about 500 oocytes at various developmental stages, although only a small percentage was smooth and located in the oviduct, ready to be laid—suggesting a multiple spawning strategy. In the Needham's sac of mature males were counted up to 146 spermatophores (35 ± 24) with an average length of 17.9 mm and large sperm mass.To ensure reproductive success in rapid and fleeting matings and in absence of a seminal receptacle, intradermal spermatangia (SPG, inverted spermatophores) implantation represents an efficient way to allow sperm storage in the body of females, even not mature yet. Moreover to this is associated the ability of the SPG to move and pass from the external of the mantle to the inner cavity to fertilisate the oocytes. Mated females analysed carried up to 33 SPG, predominantly (76%) located in the anterior ventral area above the oviduct—an optimal site for fertilization. However, spermatangia were also found on the head, funnel, and arms, likely due to errors during rapid mating. Given the presence of spg implanted in males, we can hypothesize that a mating error may concern also a mating between males.

Living in marinas and ports: underwater noise pollution effects on the behavior and physiology of the ascidian *Botryllus schlosseri*

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Marinas and ports represent extreme environments for sessile animals, which cannot escape the variety of anthropogenic pressures characterizing them, including underwater noise generated by maritime traffic. The latter is considered a pollutant of concern in the European Marine Strategy Framework Directive, being pervasive in our basins. However, the marine invertebrate ability to cope with it is mostly unknown. Here, we present our results on the effects of anthropogenic underwater noise on the colonial ascidian *Botryllus schlosseri*, a species commonly found in shallow waters of marinas and ports. We exposed colonies, sampled in the Venetian Lagoon close to Chioggia (Italy) to continuous and discontinuous noise (peak bands 63-125 Hz), mimicking the low frequency maritime traffic noise. After measurements of the lagoon soundscape, we tested noise levels (138.36-163 dB) comparable to those produced by boats passing close to Chioggia. Colonies underwent both short-term (30 min, continuous noise) and long-term (3 days, discontinuous noise) exposures. Some were also primed by noise, exposing them to a noise pulse before the noise exposure, to verify if priming could enable them to subsequently tolerate noise treatment better. To verify the effects induced by the treatments, we used behavioral assays (verifying the animal ability to detect mechanical stimuli), and physiological assays (verifying the heartbeat frequency, also over time, and the filtration rate). The expression of stress related genes, such as *sod*, *c3*, *rbl*, *catalase* (quantified by qPCR), and the immune system functionality (phagocytosis activity assays at FACS) were evaluated. After long-term exposures, we also verified the effects at developmental level, considering the asexual cycle progression and the ability to regenerate excised portions of the colonial tunic and its circulatory system. Moreover, we analyzed the possible noise transgenerational effects by exposing colonies to a long-term treatment and evaluating at behavioral and physiological level its effects on two generations of buds, once become adults. Results show that noise has negative effects on behavioral, physiological, immunological and developmental levels, inducing stress-related responses. The study evidences the necessity to monitor this pollutant for reaching the Good Environmental Status of European basins.

Transfer and physiological effects of microplastics in soil invertebrates

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The presence of microplastics (MPs) and nanoplastics (NPs) in terrestrial and aquatic ecosystems has become a significant concern, as they are a novel and pervasive form of pollution. The generation of plastic waste is a consequence of a number of human activities, including agricultural practices (e.g. plastic mulching, sewage sludge). In terrestrial ecosystems, MPs/NPs enter the food web through the root and leaf systems of plants and then reach primary and secondary consumers. However, the dynamics of microplastic transfer trough the soil trophic web remains poorly understood, as well as their sublethal effects on the ecological functions of terrestrial invertebrates. To investigate the trophic transfer of polystyrene (PS-MPs), a simplified prey-predator system was studied using *Cantareus aspersus* (Müller, 1774) snails as primary consumers and the helicophagous larvae of *Carabus lefebvrei* (Dejean, 1826) (Coleoptera, Carabidae) as predators. Second and third instar larvae were fed snails previously exposed to either 0.01% or 0.1% PS-MPs, which had been labelled with europium to be detected in tissue by ICP-MS (Inductively Coupled Plasma Mass Spectrometry). The results showed a dose-dependent accumulation of microplastics in both snails and the beetle larvae. No statistically significant differences were observed between larval stages in terms of total MP accumulation (p ¿ 0.05), suggesting that developmental stage did not influence uptake. However, the detection of MPs in exuviae collected during the moult from the second to the third larval stage suggests the existence of an efficient detoxification mechanism in the preimaginal stages of this carabid species, enabling partial elimination of ingested particles. In addition, larvae fed on contaminated snails exhibited significant increases in phenoxidase enzyme activity compared to the control group (p¡0.05 for the 0.01% and 0.10% PS-MP groups, respectively), indicating a sublethal exposure effect on immune system function involved in melanisation processes. In conclusion, this study provides new insights into the trophic transfer of microplastics in soil food webs, demonstrating both physiological effects and potential detoxification strategies in a ground predator species. These findings highlight the need for further ecotoxicological assessment of plastic pollution in terrestrial ecosystems, particularly regarding its impact on invertebrate immune function and sublethal effects on wildlife.

Reproductive strategies of the bamboo coral *Isidella elongata*: crucial data for the conservation of deep-sea corals

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The bamboo coral *Isidella elongata* (Esper, 1788) is an octocoral crucial to deep-sea ecosystems. Nearly endemic to the Mediterranean, it typically resides at depths of 500–1656 m, forming communities on soft bathyal seabeds. Classified as Critically Endangered on the IUCN Red List in both the Mediterranean and Italian waters, it is also an indicator species for Vulnerable Marine Ecosystems according to the General Fisheries Commission for the Mediterranean Sea. Despite its ecological importance, no reproductive information existed for *I. elongata*. This study aimed to investigate its reproductive biology, including reproductive mode, gametogenesis trends, polyp fecundity, sexual product (SP) development, and size at first reproduction.

Between June and September 2024, 68 colonies of *I. elongata* were collected off the southwestern coast of Sardinia at unusual depths of 160–187 m during trammel net fishing for the spiny lobster (*Palinurus elephas*). The basal diameter (BD) and height (H) of each colony were measured, and histological analyses were performed on randomly selected polyps to determine sex and maturation stages of male and female SP. A total of 2175 polyps were dissected to estimate relative fecundity, calculated as the number of SP per polyp.

All colonies were gonochoric, at both the colony and polyp levels. Sex-ratio was biased toward females, with no significant differences. On average, female polyps contained 2.58 SP, with a maximum of 24 in one polyp, while male polyps contained 7.61 SP, with up to 28 SP in one polyp. All female colonies (BD: 1.9–3.7 mm; H: 25.6–63.2 cm) were sexually mature, with SP at late vitellogenic stages. Most male colonies (BD: 2.0–4.6 mm) were in maturing or mature stages. The smallest female colony (BD 1.9 mm) had mostly empty polyps, with a few containing 3–4 mature SP, while the smallest male colony (BD 2 mm) had polyps filled with mature SP. This study provides the first reproductive data on this species. The gonochorism observed is typical for octocorals. The absence of planula larvae suggests *I. elongata* reproduces via broadcast spawning, with large female SP indicating a lecithotrophic larval type. The findings show early sexual maturity and a potentially high reproductive output. Gaining deeper insight into the reproductive strategies and cycles of deep-sea corals like *I. elongata* is essential to improve our understanding of these key habitats and to guide future research and conservation efforts.

Growth of bamboo coral *Isidella elongata* (Esper, 1788): an example of the adaptation to the deep environment in Mediterranean

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The General Fisheries Commission for the Mediterranean (GFCM) recognises bamboo coral forests as Vulnerable Marine Ecosystems due to their high ecological importance (e.g. habitat-forming, hot-spot biodiversity). The major cause of bamboo coral vulnerability lies in their slow growth rate, which limits their ability to recover from fisheries-related damage. Despite the need to take action to ensure the conservation of the *I. elongata* forests, crucial information regarding life-history traits such as age, growth rate, and life span is still limited. This study takes advantage of the incidental catch of 751 *I. elongata* colonies (basal diameter 0.36-7.3 mm) through experimental trawl fishing surveys (2012-2023) in South-Adriatic Sea. We modelled the species' growth through thin sections (basal diameter < 2 mm) and radiocarbon dating (basal diameter > 4 mm). The estimated ages, considering both methods, ranged between 1 and 59 years, while the species' lifespan in the area was predicted to be from 57.5 to 59 years. Age data were fitted to seven different growth models, with the exponetial model proving to be the most accurate in describing the species' growth with significant difference between two groups of age data (< 12 and > 48.8 years). This difference could be linked to the branching growth pattern of *I. elongata*, since young colonies are unbranched, while both the number and the size of ramifications increase with the colony age. A higher number of branches means a greater number of polyps and implies a greater feeding capacity, supporting the higher growth rate that we observed in older colonies. Fan-shaped branching might also impose biomechanical demands to resist current-induced stress, potentially limiting axial growth but, at the same time, needing a larger and more robust stem to support the colony, thus stimulating BD growth. On the contrary, it is expected that sexual maturity would lead to a decrease in growth rate, as energy is diverted from growth to reproduction. The observed exponential growth pattern may result from a trade-off adaptation among increased feeding capacity (positive effect), structural constraints (both positive and negative effects) and sexual maturity (negative effect). Our findings are the first providing direct ageing of *I. elongata* in the Mediterranean Sea, which is a critical aspect in determining the species' long-term population dynamics and recovery capacity from an ecosystem-based fishery management perspective.

The desiccome of the tardigrade *Bertolanius volubilis*

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Anhydrobiosis, a reversible metabolic state enabling survival without water, is a key adaptation in tardigrades, involving a molecular toolkit that includes Tardigrade Disordered Proteins (TDPs). Three TDP families, Cytosolic (CAHS), Secretory (SAHS), and Mitochondrial (MAHS) Abundant Heat Soluble proteins, contribute to structural stabilization, oxidative stress resistance, and biomolecule protection. Despite their role, TDPs show considerable diversification and remain poorly studied in rare clades like Eohypsibioidea, which includes few species mainly found in polar and alpine regions. Within this group, *Bertolanius volubilis* is known to survive extreme conditions through multiple dormancy strategies. However, due to its limited distribution, molecular data on anhydrobiosis are lacking. To address this gap, the transcriptomic response to desiccation in *B. volubilis* was investigated, providing novel insights into this clade.

A population of *B. volubilis*, collected from the Apennines in 2007 and stored dry for 18 years, was used. Desiccation tolerance was assessed by a rehydration survival assay. RNA was extracted from four groups of specimens in hydrated and desiccated states. The transcriptome was *de novo* assembled and analysed to identify the repertoire activated during desiccation, focusing on TDPs expression and diversity.

B. volubilis showed high survival upon rehydration. Transcriptomic analyses revealed differentially expressed genes, many lacking homologs in other tardigrades, suggesting they may be unique to this clade. Several CAHS genes were highly and constitutively expressed, with moderate upregulation during desiccation, while one SAHS gene showed stable high expression in both states. Evolutionary comparisons revealed sequences similar to known TDPs and novel variants, highlighting lineage-specific adaptations.

Results confirm *B. volubilis* as a desiccation-tolerant species and provide the first omics data for Eohypsibioidea. Its transcriptomic profile, marked by high baseline CAHS expression and limited desiccation-induced modulation, suggests a constitutive strategy for coping with rapid dehydration, as observed in other desiccation-tolerant tardigrades.

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New insights into the adaptive molecular evolution of a bipolar ciliate, *Euplotes nobilii*

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The biological concept of "metapopulation" (i.e, a group of populations of the same species separated by space but linked by dispersal and migration) applies properly also to protists' biogeography, as originally fostered by Finlay and Fenchel (2004) with particular reference to ciliates. By sampling ciliates over many years from coastal marine waters of Antarctic, Patagonian and Arctic sites, we realized a wide collection of strains representing heterospecific psychrotrophic and psychrophilic populations of *Euplotes*, an extremely diversified genus that stands at the forefront of experimental ciliatology. Among these strains, those representing *E. nobilii* have more profitably been used to improve our knowledge of the molecular adaptation of single-celled organisms to the extreme environmental conditions of polar waters. Following evidence from genetic and breeding analysis that they effectively represent geographically separated populations of the same panmictic metapopulation, a dozen of them (growing better at 4 °C at a stable rate of one fission every other day) were chosen to determine the structures of their water-borne pheromones (cell type-specific proteins that preside self/not-self cell-cell interactions) and get insights on the molecular basis of the cell defense against UV radiation and protein oxidation. In comparison with its sister species, *E. raikovi*, that is widespread in temperate sea waters, *E. nobilii* (i) synthesizes less thermostable pheromones characterized by a marked reduction of regular helical structures and a consequent extension of random coil regions (functional to improve structural flexibility), and (ii) reinforces its enzymatic barriers to UV and oxidative damages also utilizing chaperons and antioxidant enzymes (including methionine sulfoxide reductases responsible for reducing methionine sulfoxide back to methionine) which are continuously released into the cytoplasm by endosymbiotic *Parafrancisella* bacteria that appear to be pervasive colonizers of *E. nobilii* cells.

Sessione
La vita negli ambienti estremi

Poster

Changes in diversity and structure of sponge communities of a semi-submerged sea cave over a half-century time span

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Sea caves are vulnerable ecosystems and represent endangered habitats in Mediterranean Sea because of pollution, thermal anomalies and presence of alien species in recent decades. These environments offer optimal physical conditions that lead to a high level of biodiversity within a limited area. The study of the semi-submerged sea caves provides information about environmental stability on a temporal scale and changes in benthic communities on a local scale. Porifera are very common in these habitats in terms of diversity and abundance and represent a key group for assessing the effects of the possible environmental changes. This study compares the diversity of sponges over a span of time of 63-year (1960-2023) in a semi-submerged cave called "Grotta delle Palombe" at Baia di Trentaremi (Naples), located within the MPA "Parco sommerso di Gaiola". The opening of the cave is narrow and elongated, becoming even narrower toward the end, approximately 60 meters from the entrance. The same sampling method was used as in previous studies by professor Sarà. All the specimens present in standard squares (400 cm2 each) were collected by scuba diving. The number of identified species increased from 69 in 1960 to 72 in 2023, with only 27 species in common. Sponge abundance have also changed: in 1960 *Leucetta solida* (Schmidt, 1862), *Cliona viridis* (Schmidt, 1862) and *Phorbas fictitius* (Bowerbank, 1866) were the most abundant species in the cave. Instead, in 2023 *Dendroxea lenis* (Topsent, 1892), *Jaspis johnstonii* (Schmidt, 1862) and *Leucosolenia* sp.1 predominate. In 1960, *C. viridis* (14,56%) and *G. cydonium* (11,05%) were the most abundant species in terms of coverage, whereas *D. lenis* (6,11%) and *J. johnstonii* (2,5%) were the most abundant in 2023. Total sponge coverage is now around 25%, compared to 55% more than 60 years ago. Anthropogenic impact, together with ongoing climate change, have contributed to these variations, reflecting sponge adaptability to environmental changes.

Changes in meiofauna community during a coral bleaching event in the Maldives

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Coral bleaching represents one of the main threats to tropical marine ecosystems worldwide, with significant impacts also observed in the Maldives archipelago. One element that remains relatively understudied in this context is the effect of marine heatwaves on benthic meiofauna associated with coral reefs, which performs crucial ecological functions within reef sediments, including bioturbation and nutrient recycling. Recent studies suggest that the composition and abundance of meiofauna are strongly influenced by coral health: the loss of three-dimensional habitat and trophic resources following coral bleaching and subsequent mortality can lead to a decline in the diversity and functionality of these communities. The change in benthic meiofauna composition and abundance during the 2024 bleaching event was therefore investigated. Sediment samples were collected by scuba diving at two depths (1m and 8m) in Magoodhoo lagoon (Faafu Atoll, Maldives) before (April 2024), during (July 2024), and after the bleaching event (December 2024). Meiofauna was extracted, counted and sorted to the main taxa level. A total of 8 taxa were recorded of which the most abundant were Nematoda and Copepoda with their nauplii. The highest abundance (1075±388 individuals) was observed at the 1m station before the bleaching event, while the lowest (399±149) was at 8m in the same period. Community composition varied significantly before, during and after the bleaching event. In particular, the relative abundance of Nematoda was markedly reduced during and after the bleaching event compared to pre bleaching values suggesting potential influence of thermal stress during the bleaching event or seasonal dynamics. Conversely, samples from during and after bleaching event showed higher proportions of Copepoda and their nauplii, potentially indicating reproductive events during this period. Depth also played a role in shaping community structure, with deeper stations exhibiting lower abundance. These patterns may reflect environmental changes, such as temperature fluctuations, oxygen levels, and food availability. Our results show coral bleaching events not only affect coral but may also impact meiofauna associated with corals. Understanding the interactions between bleaching events and meiofaunal communities is critical for assessing reef ecosystem resilience and informing targeted conservation strategies in the Maldives, a region particularly vulnerable to the effects of climate change.

Antarctic gastropods and algal turf: taxonomic composition, distribution and ecological interactions in shallow water of Terra Nova Bay (Ross Sea)

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The structure of marine benthic communities is primarily shaped by environmental variables, especially physical factors such as sediment type, hydrodynamics, and oxygen availability. In hard-bottom and algal-dominated habitats, the nature and complexity of the substrate play a key role in determining species composition through physical constraints and biological interactions. In shallow water of Terra Nova Bay (Ross Sea, Antarctic continent), macrofauna was sampled from hard substrate, in the austral summer 2021/2022, during the XXXVII Italian Antarctic expedition of the "National Program of Antarctic Research" (PNRA). Samples, from hard substrates both colonised and not colonised by high algal turf, were collected using an air-suction pump in a frame of 40x40cm. The gastropod assemblages were taxonomically investigated, and algal turf biomass was quantified. *Laevilitorina antarctica* (Smith, 1902) and *Powellisetia deserta* (E. A. Smith, 1907) were the dominant species, accounting for 68% of the total gastropod assemblages, followed by *Subonoba turqueti* (E. Lamy, 1906), which contributed 16%. The presence of algal turf strongly influenced gastropod distribution, with 81% of individuals found on algal-colonised substrate, and only 19% on non-colonised ones. This pattern was statistically supported by the linear regression analysis, which, considering algal biomass as the independent variable, revealed a strong positive relationship between algal turf with gastropod abundance (R2=0.94; p¡0.001), whereas no significant relationship was detected with species richness (R2=0.0008; p¿0.05). These results reinforce the ecological role of habitat-forming species, such as erect algae, acting as ecosystem engineers. The dominance of a few species suggests that algal turf substrate is highly selective for certain *taxa*, highlighting the importance of structural complexity in enhancing habitat suitability. Overall, in extreme environments, such as the Antarctic continent, turf-forming algae significantly influence gastropod assemblages' composition and spatial distribution.

Revisiting diversity and endemicity of deep Mediterranean polychaetes: occurrence of a *Nereis* clade associated with reduced environments on Tyrrhenian volcanoes

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Recent studies carried out in the southern Atlantic Ocean highlighted the occurrence of four *Nereis* species sharing some morphological features and structuring a supported clade from the molecular point of view. These species have been found in association with experimental organic falls, suggesting adaptation to deep-sea reduced environments. Samplings carried out in 2018 on the submarine volcanoes of the southern Tyrrhenian Sea highlighted the occurrence of an eyeless *Nereis* species associated with hydrothermal vent environments. Morphological and molecular data support its inclusion in the southern Atlantic clade, but also suggest that it represent a distinct, undescribed species. Comparison of the sequences obtained with those publicly available on genetic databases stressed a close similarity with specimens sampled on mud volcanoes in the Gulf of Cadiz, misidentified as *Eunereis longissima*, suggesting that this clade of Nereis might be rather widespread in deep European waters. These results support the hypothesis of the occurrence of a Mediterranean fauna associated with deep hydrothermal vents and more generally reduced environments, and the possibility that some of these species might indeed be endemic to the Mediterranean Sea.

Bluecrab Interreg: the reproductive biology of ***Callinectes sapidus*** in Caorle and Marano lagoons

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The blue crab *Callinectes sapidus* (Rathbun,1896) is an invasive alien species in the Mediterranean Sea. In recent years, its adaptability allowed it to spread rapidly, posing a significant threat to local biodiversity and traditional fisheries,particularly along the northern Adriatic coast. This study was carried out within the Interreg ITA-SLO –BLUECRAB Blue Crab Lifecycle Understanding and Ecosystem Management in Europe (ITA-SI0600229), which aims to protect vulnerable areas of the upper Adriatic through monitoring and sustainable management involving institutions, fishing companies and local communities. Despite its growing impact, limited information is available about the species' local life cycle and reproductive biology, knowledge that is essential to implement effective control measures. To address this gap, monthly crab samplings were conducted in the Marano and Caorle lagoons at three stations characterized by increasing salinity gradients. In Marano, specimens were catched using a fyke net, whereas in Caorle, two traditional traps were employed: a square one and a round one. At each station, a multi-parameter probe was placed to record temperature and salinity, which are known to influence the species' distribution and behaviour. Carapace width, carapace length and body weight were measured for each individual. A subsample of 20 crabs per lagoon was dissected to assess the gonado-smatic index and hepato-somatic index, as well as to examine gonadal development. Tissues were embedded in resin for histological analysis to confirm reproductive stages. In total, 297 crabs were sampled. In Caorle, the average size is 147.8±28.8mm for females and 152.6±19.7mm for males, while in Marano 171.5±9.8mm for females and 176.2±13.8mm for males. Mean weights are 158.4±56.2g (females) and 229.8±84.7g (males) in Caorle, and 229.0±51.8g (females) and 372.7±71.7g (males) in Marano. The M:F sex ratio is 1:0.79 in Marano and 1:0.82 in Caorle. Preliminary findings indicate a reproductive season extending from May to August, with peak between June and August. Analysis of reproductive indices is ongoing, with the aim of characterizing the timing and duration of ovarian development and identifying reproductive migrations. This study represents the first investigation into the reproductive biology of *C. sapidus* at higher latitudes within the Mediterranean. Initial data suggest distinct physiological responses, potentially linked to local environmental conditions.

Brain evolution in Mediterranean Sea sharks: unveiling adaptive ‘cerebrotypes’ for different environments

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Chondrichthyans occupy a basal position in vertebrate evolution, providing a relatively unexplored opportunity to study the evolution of vertebrate brains. This study examines the brain morphology of 12 shark species and one holocephalan, all representative of Mediterranean species, in relation to their habitat (bathyal, coastal, or pelagic), hypothesizing a contrast between deep-sea and shallow-water/pelagic brain models.

The relative development of the five major brain areas (olfactory bulbs, telencephalon, diencephalon, mesencephalon, cerebellum, and medulla) was assessed, comparing their percentages across the three habitats and involving PCA analysis. Since the olfactory bulbs are known to evolve statistically independently from the rest of the brain, PCA analysis was performed both with and without the olfactory bulb percentages.

The results show that deep-sea sharks and holocephalans share common characteristics, including a relatively reduced telencephalon, a smooth cerebellar corpus, and an extreme relative enlargement of the medulla, which may reflect adaptations to their specific deep-sea environment. The reduced telencephalon suggests a lower need for advanced cognitive functions, while the smooth cerebellar corpus could indicate an emphasis on energy efficiency rather than fine motor control. The enlargement of the medulla likely supports vital autonomic functions crucial for survival in extreme conditions, as well as the modulation of electroreception and lateral line cues. The lack of habitat-specific development of olfactory bulbs suggests that olfaction may not be as critical or may be subject to evolutionary constraints unrelated to habitat.

Although not a functional analysis, the observed traits suggest the Mediterranean deep-sea Chondrichthyans possess peculiar brain patterns, shaped by both evolutionary history and their adaptation to harsh conditions, reinforcing the concept of ‘cerebrotypes.’

The BIOROSS 2023 Project: increasing knowledge of the sponge fauna of the Ross Sea (Antarctica)

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The Ross Sea (Antarctica) has been the interest of several Italian scientific expeditions since 1958. During these campaigns, the study of benthic communities, and in particular Porifera, has been one of the main focuses of research on the area's biodiversity. The most recent expedition took place in 2023 as part of the BIOROSS Project, led by ENEA and funded by MUR-PNRA. Researchers from DISTAV (UNIGE) were in charge of analysing sponge samples from 13 sites located between Cape Wheatstone and Robertson's Bay, collected by Agassiz trawl at a depth range of 50-500 m. A total number of 113 specimens belonging to the class Demospongiae have been collected, among which 42 species have been identified. Most of the specimens were collected at two sites, offshore Cape Wheatstone and along the coast of the Hallet Peninsula, with 42 and 26 samples, respectively. The most represented and diversified genus was *Isodictya* Bowerbank, 1864, of which 30 specimens were collected, belonging to the following 9 different species: *I. cf. delicata* (Thiele, 1905), *I. erinacea* (Topsent, 1916), *I. kerguelenensis* (Ridley & Dendy, 1886), *I. lankesteri* (Kirkpatrick, 1907), *I. setifera* (Topsent, 1901), *I. spinigera* (Kirkpatrick, 1907), *I. toxophila* Burton, 1932, *I. trigona* (Topsent, 1917), and *I. verrucosa* (Topsent, 1913). Moreover, *Isodictya* was the genus with the widest distribution, occurring in 8 out of the 13 sites. Among the identified sponge species, 5 were new records for the area of the Ross Sea, as follow: *Phorbas megasigma* Rios & Cristobo, 2007, *I. cf. delicata*, *I. trigona*, *Ectyonopsis ruthae* (Mothes & Lerner, 1995), and *Antarctotetilla pilosa* Carella & Uriz, 2018. Furthermore, *E. ruthae* and *I. trigona* were found for the first time after the original description. Finally, the species *Microxina sp.* and *Halichondria (Halichondria) sp.*, probably represent two species new to science. This study has therefore broadened knowledge of the sponge fauna of the Ross Sea, increasing the number of known species from 264 to 271, thus improving the understanding of its biodiversity.

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Sky polarization gradient and zonal orientation in young sandhoppers

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It is known that the supralittoral amphipod *Talitrus saltator* (Montagu) uses various celestial orientation factors during daily movements along the sea-land axis of the beach to avoid biotic and abiotic stressors. The sun compass mechanism is one of the main orientation mechanisms used and is innate in young sandhoppers. However, although it has been shown that the sandhopper *T. saltator* perceives polarized light and possesses an arrangement of the rhabdomeres that could allow e-vector utilization, *T. saltator* doesn't use the e-vector orientation of the skylight polarization as a compass cue. However, adult individuals perceive radiance and/or color gradients more effectively when exposed to polarized light. In the laboratory experiments were carried out with young sandhoppers collected in the field over a month old (experts) and laboratory-born sandhoppers of varius ages (inexperts) under an artificial linear polarization gradient. Experts sandhoppers use the linear polarization gradient to steer in the correct direction of the sea-land axis of the original beach, whilst inexpert young born in the laboratory are unable to take any direction independently of their age. However, the latter after a training period in the laboratory under the polarization gradient and a false sun are able to head in the correct sea-land axis even if tested under the sole polarization gradient

Sessione
Approcci molecolari nella zoologia contemporanea

Comunicazioni

Shark tales – the stories told by molecules

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Elasmobranchs (sharks and rays) are charismatic vertebrates that capture the public´s attention around the world. This ancient group of jawed vertebrates dates back 350 Myr, predating the dinosaurs, and surviving several mass extinction events. Currently, overfishing, habitat degradation and climate change have led to population depletion and to ˜1/3 of extant elasmobranchs being threatened, while many are still Data Deficient. Indeed, studies based on direct observation of shark and ray populations are logistically challenging, costly and may induce individual mortality (e.g. fishing). Here I provide several examples of how molecular genetics, coupled to non-invasive or minimally invasive sampling, may provide important clues on elasmobranch biology, ecology, diversity and evolution, with important management and conservation implications. Specifically, molecular genetic studies have helped uncover important aspects of reproductive biology and behavior, such as the diversity of mating systems in elasmobranchs. On the other hand, population genetics and genomics have also helped clarify the main patterns and drivers of population structure of sharks and rays, and to identify the putative barriers to gene flow in different taxa. Molecular markers have also greatly improved species identification as well as detection of cryptic species, which are essential to accurate data collection and analysis. Technological advances in genetic data collection, namely next generation sequencing, have expanded the scope of the questions and the fraction of the genetic material available for interrogation. For instance, the number of high-quality, complete whole genomes for elasmobranchs have opened the way to comparative genomics and the opportunity to look at the evolution of jawed vertebrates and their unique features (e.g. an adaptive immune system) from the perspective of one of their most ancient and basal group – the Cartilaginous fish. Finally, the field of environmental DNA analysis offers the possibility to survey and monitor species diversity in time and space in a non-invasive way, which is particularly important in the case of the highly elusive and endangered elasmobranch taxa. I conclude with examples of knowledge gaps in elasmobranch ecology, diversity and evolution, where molecular tools can lead to major breakthroughs.

Recovery of past species diversity and historical ecology of sawfish rostra in the Mediterranean museums and collections

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Sawfishes represent one of the most critically endangered groups among elasmobranchs and have experienced local extinctions across numerous coastal regions worldwide. Contemporary conservation strategies increasingly highlight the importance of reconstructing historical changes in species distributions and geographic ranges. Within the Mediterranean, such reconstructions have indicated the historical presence of two sawfish species, presumed extinct since the 1970s, challenging earlier assumptions about their biogeographical range.

In this study, we investigated whether historical sawfish specimens preserved in various institutional and private collections could provide evidence of a Mediterranean origin. A total of 229 rostra were examined, including 28 specimens labelled as originating from the Mediterranean region. Most of these samples lacked precise taxonomic classification and robust collection metadata. We performed morphometric analyses alongside mitochondrial DNA sequencing (targeting partial COI and NADH2 regions) achieving the assignment of all rostra to four extant species: *Pristis zijsron* (104 specimens), *Anoxypristis cuspidata* (52), *P. pristis* (47), and *P. pectinata* (26). Among the rostra labelled as Mediterranean, we identified *P. zijsron* (9), *A. cuspidata* (8), *P. pristis* (6), and *P. pectinata* (5).

Following taxonomic identification, we implemented a preliminary isotopic approach to infer historical provenance, based on stable isotope analysis (^{13}C and ^{1}N) of rostral teeth and collagen-rich tissues from the rostra. Initial results revealed significant ^{13}C variation between specimens from the Red Sea and those from other documented localities. Furthermore, high ^{1}N values were observed in many A. cuspidata individuals, suggesting associations with nutrient-rich, upwelling-influenced coastal environments. The Mediterranean-labelled rostra remain tentative and require further validation through a comparison with historical baseline for elasmobranchs we are building up using the “P. Doderlein” osteological collection. These findings underscore the importance of integrating museum collections and stable isotope analyses to refine our understanding of sawfish natural history, establish historical population baselines, and could enhance global conservation planning.

Molecular tools to gain a deeper knowledge on Sardinian Chondrichthyan species

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In the marine environment, chondrichthyan species—which include sharks, rays, skates, and chimeras—are one of the most endangered taxa. Being among the slowest-reproducing vertebrates in the sea, they are extremely vulnerable to anthropogenic pressures such as overfishing, habitat modification, pollution, and climate change. Innovative molecular methods can unlock new possibilities to explore their distribution and to monitor their diversity over time. Genetic tools can especially help in addressing the challenge of precisely identifying species, a difficult task in a group characterized by a high morphological conservatism, but essential for developing effective conservation and management strategies.

In this context, we applied a genetic approach to deepen our knowledge of Mediterranean chondrichthyans, with a special focus on the seas around the Sardinian Island (Western Mediterranean). A few case studies are described here, based on data collected during the building of a curated multi-marker reference library based on mitochondrial genes (COI, ND2, and 12S).

In particular, we investigated the electric rays, observing the presence of potential cryptic species within the order Torpediniformes and measuring the intraspecific diversity of the three Mediterranean species (*Torpedo torpedo*, *Torpedo marmorata*, and *Tetronarce nobiliana*). Similarly, the application of several 'species-delimitation' methods allows us to find evidence of taxonomic uncertainties in the five genera of Mediterranean stingrays (*Bathytoshia*, *Dasyatis*, *Himantura*, *Pteroplatytrygon*, and *Taeniurops*), suggesting the urgent need for future studies and a comprehensive revision of the family Dasyatidae for its effective conservation. Moreover, sequence data allowed us to confirm the occurrence of *Dasyatis tortonesei* in the investigated area and to highlight that misidentification with the congeneric *Dasyatis pastinaca* can easily occur, suggesting the urgency for new morphological identification keys. Finally, the curated reference library allowed us to further update the Sardinian cartilaginous fish species checklist with a rare species (*Rostroraja alba*) and an endemic species (*Leucoraja melitensis*), expanding their known geographic range of distribution.

Mitochondrial genomes: a resource for Tardigrade phylogenetics

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Mitochondrial genomes (mtDNAs) are well documented in many metazoan taxa and widely applied to evolutionary and phylogenetic studies. In tardigrades, however, mtDNA data remain limited, and their potential in phylogenetic analyses is still poorly assessed. Although the cox1 gene is routinely used for species delimitation and population studies in tardigrades, other mitochondrial genes remain understudied in terms of sequence variability and phylogenetic utility. Additionally, knowledge on mtDNA synteny (i.e. gene order conservation) within Tardigrada and its possible links to ecological adaptation and stress resilience is lacking. Therefore, the aims of this study were to expand mtDNA data for tardigrades and assess their usefulness for phylogenetic reconstruction and comparative genomic analyses.

We examined 23 mitochondrial genomes from tardigrades spanning multiple habitats, cryptobiotic capabilities, and phylogenetic lineages. Genomic DNA was isolated from single individuals using either Total Genomic Extraction or Whole Genome Amplification, followed by Illumina sequencing. MtDNAs were assembled, annotated, and phylogenetically analysed through bioinformatic pipelines based on both concatenated nucleotide and aminoacid sequences. We also investigated mitochondrial gene synteny across tardigrade species.

Tardigrade mtDNAs are approximately 15 kb in size, typically encoding 13 protein-coding genes, 2 rRNAs, and around 22 tRNAs. Phylogenetic analyses yielded strongly supported clades, with a topology consistent with trees based on 18S and 28S rRNA sequences, supporting the utility of concatenated mitochondrial genes in resolving phylum-level relationships within Tardigrada. Gene order analysis revealed a remarkable level of synteny across the group, unaffected by habitat type, environmental conditions, or cryptobiotic capabilities. This indicates that mtDNA genomic structure in tardigrades remained largely conserved throughout their evolutionary history.

MtDNAs offer strong resolution for deep phylogenetic questions and may also help clarify polytomies left unresolved by commonly used markers. This strategy is efficient, as it enables phylogenetic inference, species delimitation, and population-level analyses to be performed using high-throughput data generated from a single individual, with relatively minimal effort.

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A new look at the phylogeny of Macrodasyida (Gastrotricha) through improved taxonomic and molecular sampling

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Gastrotrichs are microscopic, free-living invertebrates inhabiting aquatic ecosystems worldwide. The approximately 900 known species are classified into two orders: Chaetonotida (520 spp.) and Macrodasyida (380 spp.). The understanding of phylogenetic relationships within both orders is rapidly advancing, fueled by the discovery of new species and the emergence of additional information. Molecular data have become essential in complementing traditional morphological analyses to resolve taxonomic groupings. Unfortunately, many taxa are still underrepresented in molecular studies, leading to uncertainties about their origin and phylogenetic relationships. For example, the family Cephalodasyidae consists of five genera grouped on plesiomorphic and negative morphological traits. Studies utilizing 18S rDNA sequences suggest that this family may be polyphyletic; however, the exact phylogenetic relationships of its genera remain unclear. Similarly, molecular analyses indicate that Macrodasyidae may also be polyphyletic, but insufficient taxonomic and molecular sampling have hindered a definitive resolution. Our study aims to refine the internal phylogeny of Macrodasyida through improved taxonomic and molecular sampling. Using a bioinformatics pipeline based on whole-genome amplification and sequencing, we obtained 63 new sequences from 21 macrodasyidan species and integrated them with published data. We analyzed the concatenated sequences of three genes (18S rDNA, 28S rDNA, COI mtDNA) from 51 terminals using Maximum Likelihood and Bayesian Inference. Our dataset includes taxa from 9 Macrodasyida families and 21 genera, alongside taxa from two families and two genera of Chaetonotida. Our findings confirm the polyphyly of Cephalodasyidae. *Dolichodasys* and *Paradasys* cluster with Redudasyidae, while *Cephalodasys* and *Mesodasys* form distinct, unrelated lineages. Notably, *Cephalodasys mahoae* is nested within *Paradasys* rather than *Cephalodasys*, suggesting an original misidentification. The phylogenetic placement of *Pleurodasys* remains uncertain. Meanwhile, Macrodasyidae is non-monophyletic, with *Urodasys* forming an independent lineage. Additionally, the first molecular data ever obtained for Dendrodasys hint that the family Dactylopodolidae is likely polyphyletic as well. Our findings emphasize the need to revise the morphological diagnosis of certain families and to establish new groups for a more natural classification of macrodasyidan Gastrotricha.

Comprehensive DNA barcoding of Italian birds

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The Italian peninsula and its Islands host about 540 bird species (287 of which breeding) for a total of 702 subspecies. More than a third are SPECs (Species of European Conservation Concern), 2% are Endangered, 9% are Critically Endangered, and 18% are Vulnerable. In addition, Italy hosts demographically important breeding populations belonging to species of global or regional conservation concern. Nevertheless, knowledge on the genetic diversity of Italian species is still lacking, as only about twenty species were deeply investigated and, before our study, the Barcode of Life Data system (BOLD) hosted only 311 sequences belonging to 31 species and 29 BINs from Italy.

It is well known that curated reference DNA barcode libraries are fundamental not only in species-level identification but also in studies on evolution and species diversity at both small and large scales. They are also important for species identification in eDNA metabarcoding approaches, forensic analysis, and conservation projects, for the identification of Management Units (MUs) and Evolutionary Significant Units (ESUs).

As part of the BIOURBAN-IMON Project (BaC NBFC spoke 5), we started an extensive sequencing effort of the mitochondrial Cytochrome c Oxidase I (COI) region to provide a comprehensive DNA barcode reference library of the diversity of Italian birds. This work was based on the biological samples included in the two biobanks (tissues with voucher specimens and blood with biometry data) that our research group has developed since 2000s.

To date, we have obtained DNA barcoding sequences from more than 200 species of Italian birds; for each species, we have sequenced between 1 and 8 samples distributed across the Italian peninsula, as well as Sicily and Sardinia. Our data leads to an increase of more than 600% of species and 200% of sequences in BOLD. The general intraspecific variation calculated among sequences agrees with literature, but high genetic distance was found in some species, suggesting a possible phylogeographic structure.

Our data highlighted the hidden diversity in Italian species and will go on to build a comprehensive library of reference DNA barcodes that will be fertile ground for species-specific in-depth studies on the diversity of Italian and Mediterranean avian populations.

Someone has to do it! Integrative taxonomy of the sea slug *Melibe viridis* (Kelaart, 1858) (Gastropoda, Nudibranchia)

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Nudibranchia molluscs are a group of highly specialized gastropods characterized by the complete loss of the shell in the adult stage. These shell-less molluscs show an extremely variable body shape that can reflect their specialized adaptive habits. In this framework, species belonging to *Melibe* genus are one of the most specialized, with adaptations involving swimming ability, symbiotic relationships and feeding behaviour. Thanks to its modified body, *Melibe viridis* (Keelart, 1858) is capable of actively swimming and feeding through sucking and filtering. This species is one of the biggest nudibranchs known so far and the only one species of its genus present in the Mediterranean basin, where it has a stable population in the Mar Piccolo of Taranto in Southern Italy (Ionian Sea). Its unique morphology allows an easy identification, and, in fact, no questions were raised so far on its species identity. However, considering the ever-increasing cases of cryptic diversity revealed in the Mediterranean Sea thanks to the advent of the integrative taxonomy approach, morphological, embryological and molecular analyses were carried out on specimens collected from different localities. Species delimitation analysis and phylogenetic reconstructions were performed using the mitochondrial COI and 16S and the nuclear H3 markers. Single gene and concatenated molecular datasets, including new sequences and others already present in GenBank, from both Mediterranean and extra Mediterranean localities, were used to explore the ranges of intraspecific and intrageneric variability by comparison between *M. viridis* and seven additional congeneric species. Results revealed a more difficult scenario than what expected. *M. viridis* is a complex of species of which only one lives in the Mediterranean basin. In-depth morphological analysis on the characters considered diagnostic for this genus (i.e. stomach plates and reproductive system), and a deep bibliographic study, brought to light a troubled taxonomic history and helped to finally clarify the systematics of this unexpected complex of species. Mediterranean nudibranch fauna is a source of neglected and/or cryptic diversity that highlights once again the gaps still existing in the knowledge on this highly specialized group of shell-less gastropods and calls for an ever-increasing effort in zoological studies

Environmental DNA-enabled monitoring offers both opportunities and challenges for the conservation of Mediterranean elasmobranchs: lessons learned within the ELASMODROP collaborative initiative

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Monitoring marine biodiversity is crucial for ecosystem conservation and fisheries management. Traditional methods such as visual surveys and capture techniques are invasive, costly, labor-intensive, and often ineffective at detecting rare or elusive species. Advances in environmental DNA (eDNA) allow researchers to identify organisms from water samples, offering a non-invasive, sensitive alternative for assessing biodiversity.

We applied eDNA-based methods to monitor elasmobranch diversity in Italian seas, with a large-scale effort across different environments, thanks to the ELASMODROP collaborative network, which unites researchers, academics, and students to advance eDNA applications in marine biodiversity monitoring. The network enabled expanded sampling, standardized methods, and a comprehensive assessment of elasmobranch diversity.

We present case studies highlighting various eDNA sampling strategies:

Five eDNA sampling systems (active and passive) were tested in a controlled environment. Active samplers yielded more DNA and detected all elasmobranch species; passive tools were less efficient. Passive samplers deployed with deep-sea longlines detected 78% of the species captured and uncovered additional pelagic and mesopelagic taxa missed by traditional methods.

Active filtration at 25 sites in the central Mediterranean using Niskin bottles at three depths allowed for detailed depth-dependent biodiversity assessments.

Over 500 samples collected along the Italian coast in 2024 using both approaches expanded spatial coverage and provided comprehensive biodiversity data.

All samples were processed by eDNA metabarcoding with an elasmobranch-specific marker. Taxonomic assignment was based on a custom 12S reference database that incorporated newly generated and curated public sequences. The accuracy was improved by increasing taxonomic coverage and integrating results from 12S, COI and NADH2 markers. Over 15% of public sequences were found to be mislabelled. Our custom database now allows reliable identification of up to 90% of Mediterranean cartilaginous fish species.

These findings confirm eDNA as a powerful tool for comprehensive marine biodiversity assessments. While challenges remain, especially in standardizing methods and interpreting data, addressing these will enhance eDNA's role as a cost-effective, large-scale tool, laying the foundation for a stronger contribution to informed conservation strategies and policy development.

Small basin, big data: eDNA metabarcoding tracks vertebrate diversity and distribution

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Effective biodiversity monitoring is vital for detecting species loss and early incursions of alien species, especially in freshwater systems under increasing pressure. Traditional monitoring methods in flowing water are often invasive, require taxonomic expertise, and involve handling organisms, which can cause mortality. Environmental DNA (eDNA) metabarcoding offers a non-invasive alternative by detecting genetic material that organisms release into their environment. In this study, we applied eDNA metabarcoding at five sites within the Serchio River basin, an understudied watershed in Tuscany, central Italy. Water samples were filtered, and eDNA was extracted from rivers with varying flow regimes. This method identified 64 taxa, including protected native and invasive alien species, across major vertebrate groups. Species distributions matched ecological expectations. We compared eDNA results with traditional monitoring techniques, including transect surveys (March 2021–October 2023) and electrofishing. eDNA metabarcoding detected higher overall species richness, though some taxa were uniquely identified by each method. Richness was typically greater in the river's mainstem than in its tributaries, likely due to eDNA accumulation, suggesting that main channels may be strategic points for efficient biodiversity monitoring. Overall, eDNA metabarcoding enabled a rapid, non-invasive assessment of vertebrate diversity in the Serchio River basin, capturing both aquatic and terrestrial species, and detected also alien species at the early stage of invasion. This approach revealed valuable insights into regional gamma diversity, and proved more time-efficient than conventional methods.

Marine benthic biodiversity shuffles and homogenizes under the effect of marine heatwave

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The global ocean has considerably warmed over the past century, with far-reaching implications for marine biodiversity and ecosystems. Over the past decade, marine heatwaves (MHWs), defined as discrete periods of extreme regional ocean warming, have increased in both duration and frequency. There is, therefore, an urgent need to understand the response of marine biodiversity to MHWs. In this study we analysed the effects of MHWs on marine benthic communities in the Western Mediterranean Sea. A combination of standardized sampling units, i.e. Autonomous Reef Monitoring Structure (ARMS) and high-throughput sequencing of mitochondrial cytochrome c oxidase subunit I, was used to measure variation in benthic biodiversity in two sampling sites (Berni and Santa Caterina), characterized by different thermal regimes. Overall, we found 241 eukaryotic families belonging to 22 different phyla. The most abundant phylum was Porifera (30%), followed by Briozoa (22%) and Cnidaria (17%). Before the heatwaves, the two sites appeared to be significantly different in terms of richness and taxonomic composition, with significantly higher alpha diversity in the warmer site (21 eukaryotic taxa in Berni vs 17 in Santa Caterina). After the heatwaves, rather than the predicted collapse of biodiversity, we found statistically significant changes in the relative abundance of phyla in both sites, with a decrease of beta diversity (10% between the two sites) and an overall homogenization of taxonomic composition. Significant shuffles in assemblage structure were observed, driven by decreased abundances of less heat-tolerant taxa (e.g., Echinodermata, Nudibranchia) and increase of more heat-tolerant ones (e.g., Decapoda, Polychaeta). This study highlights that MHWs can drive biodiversity patterns with potential consequent effects on species interactions and ecosystem processes. Given that most of hard-bottom species are members of the understudied cryptobenthos and the anticipated increases in frequency of MHWs under current climate projections, our findings highlight the need to further study how marine biodiversity may response to future climate conditions.

Testing the impact of agricultural practices on protist and metazoan communities in rice paddy soils through environmental DNA

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Soil is a fundamental environment hosting a high biodiversity and enclosing numerous micro-habitats. Multiple studies have proved that the adoption of more sustainable practices in crop cultivation results in improved soil biodiversity conservation, whereas conventional agronomic practices can have detrimental impacts on soil attributes. Besides, rice is a major staple food and at the same time, the landscape heterogeneity within its agroecosystem is of fundamental need for several different organisms linked permanently or temporarily to water.

In this study, we used an environmental DNA metabarcoding approach targeting two different genes, the 18S rRNA and the cytochrome C oxidase subunit I (COI), aiming to assess the changes in the protist and metazoan soil communities and their functional diversity under three different rice cultivation managements (i.e. agroforestry, organic and conventional), and along four stages of the rice growth cycle (i.e. basal, vegetative, flowering and maturation phases).

Results showed that the most abundant phyla in the rice paddy soil were Cercozoa and Ciliophora for protists, and Annelida, Nematoda and Arthropoda for metazoan. In particular, Cercozoa were abundant in the agroforestry cropping system, while Ciliophora showed higher abundance in control cover crop field. Annelida were more abundant in the conventional cultivation regime, while both Nematoda and Arthropoda were less abundant, with a significant increase in the organic and agroforestry regimes. Considering the taxonomical and functional diversities, slight differences among treatments were identified both in protists and in metazoans because of the combined effect of agricultural management and the succession of drying-flooding phases during the growing season.

The community's beta diversity described a positive effect of the organic and agroforestry cropping systems, highly dissimilar from the community found in the conventional rice field. Soil physical-chemical properties did not differ significantly from one treatment to another. This study broadens our understanding of the effects of agricultural practices on the biodiversity inhabiting the soil in rice agroecosystems, highlighting the positive impact of organic and agroforestry management as suitable environments for the rice soil biocenosis. It also contributes to emphasizing the importance of soil biodiversity conservation and the benefits of redesigning agricultural practices.

Hunting the hidden: environmental DNA reveals Tardigrade biodiversity in leaf litter

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Tardigrades are abundant micrometazoans in soil ecosystems, yet their biodiversity is overlooked due to labor-intensive available methods. The eDNA metabarcoding offers a promising alternative for detecting this hidden fauna.
The objectives of the study were: i) to evaluate in silico existing primers and, if necessary, design new ones, ii) to test old and new primer sets on leaf litter samples, iii) to compare the biodiversity DNA metabarcoding results with those from integrative taxonomy, iv) to compare the tardigrade communities from different types of leaf litters.
We assessed existing primers for tardigrade COI, ITS2, and 18S genes through in silico analysis and designed new ones (OBITools). Primers were tested via real-time PCR on DNA from tardigrade and nematode bulks. Environmental DNA was extracted from leaf litter and sediment, followed by MiSeq sequencing. The integrative taxonomy analysis of nine leaf litter samples (beech, fir and mixed leaf litter) was conducted to validate the metabarcoding results.
In the literature, 9 eDNA metabarcoding primers were available for the COI and 17 for 18S rRNA genes, while no one for ITS2. One COI gene primer pair showed high taxonomic coverage and resolution for tardigrades, result that was not observed for any of the available 18S ones. Therefore, we designed two new 18S rRNA primers for eutardigrades and heterotardigrades and a new ITS2 set for heterotardigrades. The real-time PCR targeting the eutardigrade-specific region of the 18S rRNA gene amplified tardigrade DNA more efficiently than nematode DNA. Moreover, amplification curves of DNA from bulk samples and eDNA were very similar, suggesting good performance in complex systems. Around 20 species were identified through integrative taxonomy, highlighting a high tardigrade biodiversity. Almost all tardigrade species were present in all types of leaf litter; however, the variation in species abundances among leaf litter types suggested that environmental factors may influence abundance. Metabarcoding and integrative taxonomy yielded largely consistent results, with most species detected by both methods.
In conclusion, this study reviewed and evaluated the primers available in the literature and proposes new, validated alternative primer pairs for future studies of tardigrade biodiversity using DNA metabarcoding.

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Alps, Apennines and major islands: a complex network of evolutionary units in the Italian Brown Trout

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The evolutionary history of brown trout in the Mediterranean basin has been shaped by the glacial and interglacial phases of the Quaternary, which caused repeated extinction events followed by recolonization. In this context, Mediterranean islands, such as Sardinia, have assumed a crucial role as biodiversity hotspots, due to the prolonged isolation of their populations. A recent phylogenetic study of the genus Salmo based on whole-genome analysis suggested that Sardinian trout could represent a distinct species. To further investigate the genetic structure of these populations, the genomic approach of Genotyping-by-Sequencing (GBS) was used on 130 individuals belonging to nine native populations of the Mediterranean area (five from the Italian peninsula and four from Sardinia). The samples were selected to exclude individuals introgressed with the domestic Atlantic trout, in order to avoid homogenization effects and faithfully represent the genetic diversity attributable to the Italian taxon *S. ghigii*. Genetic analyses showed a clear hierarchical structure: the Sardinian and peninsular populations were divided into two distinct monophyletic groups, confirming previous studies. The application of different species delimitation methods revealed an even greater genetic complexity, identifying between 7 and 9 Significant Evolutionary Units (ESU), depending on the thresholds used. In Sardinia, three distinct ESUs were detected: one in the eastern sector and two in the south-western sector of the island, in line with data obtained from the mitochondrial DNA control region. In the Italian peninsula, instead, four ESUs were identified: one Alpine, one Adriatic Apennine and two Tyrrhenian Apennine. The genetic distances between the peninsular ESUs were greater than those estimated between two recognized species, *S. trutta* from northern Europe and *S. cettii*, both associated with the Atlantic mitochondrial lineage (AT). Finally, it is important to underline that all the populations – with the exception of the two belonging to the Alpine ESU – are significantly differentiated from each other, justifying their classification as Minimum Management Units (MU) for the management and conservation practices of this species, already seriously impacted by incorrect human manipulation.

Genetic evidence for the appenninization of alpine streams: tracing the origin of htchery-rearted Mediterranan trout in northen italy using molecular markers

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Over the past decade, the so-called Mediterranean brown trout (*Salmo ghigii*) has increasingly been used in restocking activities managed by sport fishing associations. However, little is often known about these hatchery stocks' origin, genetic composition, and breeding methods. These practices are widespread throughout Italy, particularly in the northern regions. This study aimed to reconstruct the origin and genetic characteristics of domestic Mediterranean trout stocks currently used for restocking in central and northern Italy. A total of 610 trout were analysed using molecular markers: six domestic stocks of Mediterranean trout, two samples of Atlantic trout (*S. trutta*), 25 wild Mediterranean trout populations, and two wild samples of marble trout (*S. marmoratus*). The results highlighted three key findings:
i) A widespread presence of Atlantic genetic traits in domestic Mediterranean stocks, indicating past hybridization; ii) Domestic stocks are genetically mixed, primarily derived from central and southern Italian rivers (Magra, Serchio, Fibreno, Volturno), and not representative of local wild populations; iii) Genetic traces found in Lombardy—previously interpreted as evidence of a native presence—are more likely the result of recent restocking with non-native Apennine-origin trout. These findings underscore the risk that current restocking practices pose to the conservation of native trout diversity. They highlight the urgent need for strict genetic monitoring and traceability of hatchery stocks to preserve local biodiversity and prevent the unintentional spread of non-native genetic lineages.

Evolution of desiccation related proteins in the tardigrade family Ramazzottiidae

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Tardigrades are a phylum of micrometazoan known for their ability to survive desiccation (anhydrobiosis). Research has focused on unveiling the molecular mechanisms behind their survival abilities, with a great interest in their biotechnological potential. In particular, by studying the model species *Ramazzottius varieornatus* and *Hypsibius exemplaris*, multiple tardigrade-specific gene families contributing to anhydrobiosis have been discovered. While almost all studies focused on the above-mentioned model species, evolutionary and comparative analyses of anhydrobiosis gene families are scarce. Our study is aimed to fill this gap by providing a comparative analysis of different anhydrobiosis-related gene families in the tardigrade family Ramazzottiidae.

We sequenced the genomes of 11 individuals belonging to 10 species from the family Ramazzottiidae (genera *Hebesuncus*, *Cryoconicus* and *Ramazzottius*). Single animals were used for Whole Genome Amplification coupled with Nanopore long-read sequencing. Assemblies were annotated ab initio and the relevant genes (Cytoplasmic, secreted and mitochondrial abundant heat-soluble proteins [CAHS, SAHS, MAHS] and Damage suppressor [Dsup]) were identified by blasting against a reference database of known sequences from those gene families. Genes sequences were manually curated and phylogenies were constructed using their aminoacidic sequences.

The assemblies ranged from about 50 to 100 Mb and were of good quality, despite a big range in their statistics (mean coverage range 55 – 280X, N50 range 35547 – 659307). The MAHS and Dsup were found with only one copy per species, while CAHS and SAHS experienced extensive duplications, at many points in their evolutionary history. In particular, the CAHS6 family split into four subfamilies after *Cryoconicus* + *Ramazzottius* diverged from *Hebesuncus*.

Our results indicate the WGA + Nanopore sequencing to be an extremely promising and easy approach to sequence tardigrade genomes. The CAHS and SAHS are highly dynamic genes that were involved in multiple duplication events even at shallow phylogenetic depths, which could indicate their importance in adaptations to local conditions experienced by different species. The cataloguing of different sequence variant of proteins of biotechnological interest, like the ones studied here, can help improve our knowledge about which sequence features are conserved and important for their function.

Genetic load in syntopic populations of two species of endangered Galápagos land iguanas with divergent demographic histories

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Small isolated populations represent unique opportunities to investigate how demographic histories influence the accumulation of deleterious mutations (genetic load). In this study, we focus on two syntopic populations of closely related Galápagos land iguanas - *Conolophus subcristatus* and *C. marthae*. While *C. subcristatus* remains relatively widespread across multiple islands, *C. marthae* is a critically endangered species represented by a single population occupying a very small area on the northern slopes of Isabela Island, marked by a history of long-term demographic decline. We generated low-coverage (5x) whole-genome resequencing data from 20 individuals of each of the two species to investigate the relationship between demographic history and genetic load. Variant sites were categorized by predicted functional impact to estimate the relative abundance of deleterious mutations. Genetic load was then compared between species.

We found that the two syntopic populations are genetically isolated, confirming reproductive isolation between the species. Overall genetic diversity was lower in *C. marthae* than in *C. subcristatus*, reflecting its smaller historical population size. While the ratio of derived alleles - assumed to be deleterious based on their predicted functional impact - counts at low and intermediate-effect over neutral sites was similar between the two species, *C. marthae* showed a significantly higher ratio of derived alleles count at high-effect to neutral sites. This pattern is consistent with genetic purging, a phenomenon whereby harmful recessive alleles are more likely to be exposed to selection and subsequently eliminated in small, inbred populations. The long-standing reduced effective population size of *C. marthae* may have facilitated this purging process, potentially contributing to the removal of harmful genetic variants over time.

Despite this indication of purifying selection, *C. marthae* remains at critical risk of extinction due to its extremely small population and narrow geographic range. Further validation with higher-coverage genomic data (20x) and more robust polarization of ancestral vs. derived alleles is currently underway and will be essential to confirm the observed trends and guide future conservation strategies. These findings, yet preliminary, highlight the importance of considering genetic load alongside demographic metrics in conservation genomics.

Continental-scale phylogeography of *Ixodes ricinus* inferred from complete mitochondrial genomes

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Ixodes ricinus is the most widespread tick species in Europe, acting as an important vector for pathogens affecting humans and animals. Despite its broad distribution and its ecological and medical importance, the genetic structure of this arthropod across its European and north African range remains poorly investigated. As a generalist three-host ectoparasite that feeds primarily (but not only) on mammals, *I. ricinus* has a good potential for long-distance dispersal and gene flow. Our aim was to investigate the genetic structure of the species on a continental scale, in order to identify the evolutionary dynamics that characterised the history of the species.

Here, we sequenced complete mitochondrial genomes from 472 *I. ricinus* specimens collected in 68 localities across Europe and northern Africa. Moreover, we included 59 previously published sequences (from 14 more localities) into our dataset to further expand the geographic coverage. We then reconstructed the phylogenetic relationships among the species' populations building maximum parsimony, maximum likelihood and Bayesian trees. Coalescent-based divergence time estimates were calculated for the main nodes, and pairwise distance estimates among the haplogroups were determined as well.

Our findings reveal five distinct haplogroups (A–E), with extensive gene flow reflected by limited geographic clustering at the continental, regional, and local scales. Notably, haplogroup E corresponds to *Ixodes inopinatus*, a tick described around a decade ago based on subtle morphological and molecular differences from *I. ricinus.* We detected this haplogroup in northern Africa and in few specimens from Italy, indicating long-distance dispersal events likely mediated by migratory birds, in line with previous research. The limited divergence between haplogroup E and the other four haplogroups does not support the definition of the former as a distinct species, at least from a mitochondrial DNA perspective. Divergence-time analyses indicate relatively slow mutational rates, and old splits among haplogroups and subhaplogroups.

Overall, our work provides new insights into the evolutionary history and population dynamics of this tick, evidencing the value of molecular approaches in reconstructing the patterns of population expansion and dispersal over time. Future work integrating nuclear genomic data will allow us to further refine our understanding of the genetic drivers behind the expansion of this arthropod.

One, no one, and one hundred thousand: preliminary data on mitochondrial DNA phylogeography of the genus *Euscorpius*, Thorell 1876, in Sicily and its surrounding areas

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The faunal assemblage of Mediterranean islands is the result of complex natural and human-mediated processes. In this frame, Sicily represents a paradigmatic case study. Three species of scorpions are currently known in Sicily: *Euscorpius altadonnai*, occurring in the Aspromonte (S Calabria) and Peloritani mountains (NE Sicily); *E. hyblaeus*, endemic to the Hyblean area (SE Sicily); *E. sicanus*, widespread throughout the island and some satellite islands. The first two species were recently described on phenotypical bases only. Here, we present the results of an extensive molecular characterization and phylogeographic analysis of the genus *Euscorpius* in Sicily and its surrounding territories. Overall, more than a hundred sites were sampled within the study area from 2021 to 2025. Collected scorpion samples were provisionally identified based on the morphological characters reported in literature. A fragment of the mitochondrial gene Cytochrome c oxidase subunit I (COI) was amplified from individual tissue fragments, and obtained sequences were analysed through a Bayesian Inference of phylogeny. Our results support the taxonomic status of *E. altadonnai* as a valid species, confirm its distribution across NE Sicily and S Calabria, and show the existence of sister relationships between the Sicilian and Calabrian populations of the species. *Euscorpius hyblaeus* was found in the wild only in the easternmost part of the Hyblean area, whereas elsewhere it was found exclusively within urban contexts and buildings, with several likely introduced populations. A more complex scenario was detected for those scorpions morphologically assigned to *E. sicanus*, with four different clades, constituting a paraphyletic group, allopatrically occurring in Sicilian mainland: the eastern clade (E Clade), the south-eastern clade (SE Clade), the western clade (W Clade), and the north-western clade (NW Clade). Further divergent clades encompass populations from satellite islands.

Overall, our results support the specific identity of the scorpion taxa recently described for Sicily, and better delineate their distribution. Furthermore, a strong phylogenetic structuring was observed within the morphospecies *E. sicanus.* New investigations are currently underway with the aim of defining the evolutionary history and the taxonomic rank of the observed *E. sicanus* clades.

New insights into the probing behaviour of *Aedes mosquitoes*

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After landing on the skin of a mammalian host, female mosquitoes of the *Aedes* genus perform intradermal probing, or rather start salivating and piercing the skin moving their mouthparts in a stereotypical way. While probing, mosquitoes spot blood vessels to acquire and digest blood and complete egg development. Fastening intradermal probing is crucial to shorten blood feeding, a necessary but risky step in mosquitoes' life.

We have characterised the role of Labrum Interacting Protein of the Saliva (LIPS)-2 in modulating intradermal probing in the tiger mosquito *Aedes albopictus* (Skuse, 1895). LIPS-2 is a female-specific saliva-enriched protein: knocking down its expression in female mosquitoes increased their probing time while blood feeding on human volunteers. After secretion, LIPS-2 is reabsorbed at the tip of the labrum, the mouthpart forming the food channel, where it binds Cuticular protein (Cp)19. The binding of LIPS-2 leads to a modification of the height of labral ridges, two cuticular structures located at the tip of the labrum hosting labral ridges receptors (lrr), which have been previously suggested to be proprioceptive. We have hypothesised that the Cp19:LIPS-2 interaction mediates proprioception of the labrum during probing, helping to control the movements of this stylet while searching for a blood vessel.

We have recently exploited genetic engineering, advanced imaging techniques and quantitative behavioural experiments to understand the molecular mechanisms outstanding LIPS-2 function. Aedes albopictus LIPS-2 knockout and control strains were generated in collaboration with Dr. Papathanos and their probing and feeding times, together with the amount of acquired blood, were evaluated in feeding experiments on human volunteers. *In vitro* feeding experiments were also performed exploiting the BiteOscope, which allows high resolution and high throughput analysis of mosquito feeding by automatic extraction of behavioural statistics from image sequences. Finally, scanning electron microscopy (EM) and volume EM were carried out on the labrum, with a particular focus on lrr.

Behavioural experiments were useful to explore the role of LIPS-2 in proprioception and chemosensation, while imaging analyses provided data about the morphological organisation of lrr. These analyses represent a base for a deeper and future investigation of the function of lrr in perception and of Cp19:LIPS-2 interaction in the modulation of intradermal probing.

Interspecies comparative spatial transcriptomic of mosquito male accessory glands

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In insects, seminal fluid proteins (SFPs) are secreted by the male accessory glands (MAGs) and play a pivotal role in reproductive success. These proteins influence female behaviour - most notably by stimulating egg-laying and reducing receptivity to further mating. SFP genes evolve rapidly, likely driven by post-copulatory sexual selection arising from sperm competition and cryptic female choice.

Despite their significance, the spatial organization of gene expression within the MAG remains poorly understood, with existing studies limited to a few species and selected genes. In this study, we employ spatial transcriptomics to map and compare gene expression patterns across the MAGs of five mosquito species: *Aedes albopictus*, *Aedes aegypti*, *Aedes koreicus*, *Culex pipiens*, and *Anopheles stephensi*. These species were selected based on their phylogenetic relationships, encompassing both closely and distantly related lineages. MAGs were dissected, embedded in OCT compound, and cryosectioned using a cryostat. Tissue sections were placed onto Curio Seeker tiles, which consist of a monolayer of spatially indexed beads. Following tissue placement, RNA transcripts were captured by the beads, and Illumina libraries were prepared and sequenced. The resulting sequence data were analysed using the Seeker bioinformatics pipeline and the R programming environment.

Our analysis reveals spatially distinct zones of transcriptional activity within the MAGs, corresponding to functional regions associated with seminal fluid protein (SFP) synthesis, secretion, and structural maintenance. A consistent anatomical organization emerged across species, with the presence of two main regions—an anterior and a posterior domain—observed in all examined MAGs. However, gene expression profiles showed low conservation across species, indicating substantial divergence.

Comparative analysis identified both conserved and species-specific transcriptional patterns, suggesting evolutionary differentiation in reproductive strategies and post-mating interactions. This study presents the first spatially resolved transcriptomic atlas of mosquito MAGs and the first cross-species spatial comparison, offering new insights into the molecular architecture of male reproductive tissues and the evolutionary dynamics of sexual selection in vector mosquitoes.

Temperature-driven changes in fitness and microbial associations of *Aedes koreicus*

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Over the past decades, Europe has experienced the introduction and spread of several invasive mosquito species, among which *Aedes koreicus* has gained increasing attention. First reported in Belgium in 2011, *Ae. koreicus* has since expanded its range across northern and central Europe, including successful establishment in northeastern Italy. As a recently introduced species, its ability to colonize temperate environments is thought to be linked to its higher tolerance to cold compared to other invasive mosquitoes such as *Aedes albopictus*, raising concerns about its potential impact on public health and its future spread under changing climate conditions.

Temperature is a key ecological factor influencing mosquito biology, affecting development rates, survival, reproduction, and interactions with symbiotic microbiota. Understanding how *Ae. koreicus* responds to temperature variation is essential to evaluate its ecological plasticity, predict its distribution, and assess its invasive potential.

In this study, we optimized laboratory rearing conditions for this newly introduced species and experimentally assessed the impact of constant temperatures ranging from 16°C to 32°C on key fitness parameters. We also examined the dynamics of microbiota composition, focusing on the prevalence of *Asaia*, a dominant bacterial genus in *Ae. koreicus.* Our findings indicate that temperatures between 24°C and 28°C are optimal for mosquito development and survival, whereas higher temperatures (¿28°C) negatively affect fitness. Additionally, temperature was shown to influence Asaia density, underscoring the role of environmental factors in shaping mosquito-microbiota interactions.

Together, these results provide important insights into the biological and ecological characteristics of *Ae. koreicus*, contributing to a better understanding of its invasive success. This knowledge is critical for refining predictive models of its spread and for informing targeted surveillance and control strategies in regions at risk.

The role of microbial diversity in symbiosis and development in two cnidaryan model species

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Cnidarians rely on complex symbiotic relationships to thrive in dynamic marine environments, particularly through associations with photosynthetic dinoflagellates of the family Symbiodiniaceae. While these interactions have been extensively studied in reef-building corals, the influence of microbial diversity on cnidarian development and symbiosis remains underexplored. To address this gap, we investigated the role of bacterial and fungal communities in two emerging cnidarian models: *Exaiptasia diaphana*, a benthic sea anemone, and *Cassiopea xamachana*, an upside-down jellyfish. We used high-throughput metabarcoding targeting the 16S and 18S rRNA genes to characterize microbial communities associated with both aposymbiotic and symbiotic stages of the two species. Specimens were exposed to different Symbiodiniaceae strains, including xenic, microbiome-depleted, and photosynthetic impaired cultures, to assess microbial community shifts during symbiont acquisition and early host development. Key developmental processes, such as pedal laceration in *Exaiptasia* and strobilation and asexual bud production in *Cassiopea*, were monitored under controlled conditions, alongside behavioral observations, photosynthetic performance, and physiological responses. In *Exaiptasia*, microbiome composition shifted with individual size, suggesting a microbial refinement during pedal lacerate maturation. In *Cassiopea*, strobilation occurred efficiently with xenic *Symbiodinium* and *Breviolum* cultures, whereas microbiome-depleted cultures accelerated strobilation despite higher algal densities. Notably, the photosynthetic impaired strain failed to establish symbiosis or induce strobilation. These findings underscore the dynamic and selective processes involved in symbiont and microbiome acquisition, with important implications for cnidarian physiology, development, and survival strategies.

Genomic surveillance of H5N1 Avian Influenza: evolutionary patterns and zoonotic implications

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Highly pathogenic avian influenza virus H5N1 has become a global concern due to its rapid spread in wild and domestic birds and its occasional transmission to mammals, including humans. Its zoonotic potential and ongoing evolution make it a priority for genomic surveillance and risk assessment, as avian influenza represents a significant cross-species threat with serious health and economic consequences.

In this study, we applied comparative genomics and bioinformatic analyses to investigate the genetic diversity and interspecies dynamics of H5N1, with a particular focus on its zoonotic potential. We analysed all available Hemagglutinin (HA) and Neuraminidase (NA) gene sequences belonging to the H5N1 subtype deposited in the GISAID database, obtained from both avian and mammalian hosts across diverse geographic regions. Phylodynamic analyses revealed several apparently interspecies clades, as well as multiple isolates carrying mutations known to be associated with human adaptation, particularly within receptor-binding regions.

No highly specialized or host-restricted clades were detected, and the observed genetic structure remained largely consistent with the assigned clade and lineage classifications. Selection analyses indicated no strong selective pressure on the viral segments overall; however, a slight increase in positively selected sites was observed in strains from high-density farming environments. Although this increase is not currently alarming, it exceeds the levels expected in wild populations.

Birds remain the primary host for H5N1, with migratory birds confirmed as ecological reservoirs and viral vectors between wild and domestic settings. The integration of genomic data, host ecology, and epidemiological context enabled the identification of geographic regions and viral lineages with elevated zoonotic risk, highlighting recurrent evolutionary patterns linked to host-switching events.

This study exemplifies the power of bioinformatic genetics within modern zoology, demonstrating the predictive value of genomic surveillance in detecting early molecular signals of zoonotic risk. Positioned within a One Health framework, this work contributes to the development of integrated monitoring strategies essential for timely prevention and response to future influenza outbreaks. The large-scale analysis of public genomic data demonstrates how molecular approaches can enhance predictive ecology and guide targeted interventions in human and animal health

Sessione
Approcci molecolari nella zoologia contemporanea

Poster

Unlocking a century of genetic history in *Pinna nobilis*: clues for conservation from ancient and contemporary lineages

Ilenia Azzena[1], Chiara Locci [1,2], Noemi Pascale [1,2,3], Ilaria Deplano [2], Riccardo Senigaglia [1], Edoardo Batistini [4], Daniela Caracciolo [5], Mariachiara Chiantore [6,7], Saul Ciriaco [4,8], Maria Paola Ferranti [6,7], Daniele Grech [9], Arianna Liconti [10], Monica Montefalcone [6,7], Alice Oprandi [6], Valentina Pitacco [11], Marco Segarich [4], Fabio Scarpa [2], Marco Casu [1], Daria Sanna [2]

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Pinna nobilis, the noble pen shell, is a critically endangered bivalve species which is endemic to the Mediterranean Sea. Over the past century, its populations have drastically declined due to human overexploitation, pollution, and, more recently, widespread mass mortality events (MMEs). In this study, we analysed mitochondrial COI gene sequences from both historical (1700s, 1920s, 1970s, 1990s) and modern (2000s) specimens, including individuals that survived the latest MMEs. To study the historical samples, we developed a reliable method for extracting DNA from small fragments of the byssus gland more than 100 years old. This breakthrough allowed us to explore the species' evolutionary history with unprecedented detail, thus understanding how *P. nobilis* genetic makeup has been affected by major stressors over time—namely overharvesting, environmental pollution, and climate change.

We examined a total of 667 COI sequences, 119 of which were newly generated in this study. Phylogenetic and phylogeographic analyses revealed two main mitochondrial clades: Clade A, which traces back to early ancestors around 2.5 million years ago, and Clade B, which originated from the major diversification event which involved the species approximately 1.5 million years ago during the early Pleistocene.

Despite recent population crashes, the overall mitochondrial haplotype structure of *P. nobilis* has remained stable. This suggests that specific key haplotypes—present in both ancient and modern individuals—may be associated to genetic traits that help the species to cope with environmental stress and survive over long timescales. The persistence of these haplotypes in modern surviving populations, particularly those with high genetic diversity, highlights the importance of protecting even small, groups of individuals. These groups can represent a reservoir of advantageous lineages that can guarantee the resilience of the species.

Our results offer a hopeful perspective for the conservation of *P. nobilis*, showing that its genetic legacy, probably shaped by ancient evolutionary events, still holds the potential for recovery. Our findings provide valuable insights for guiding future conservation strategies, especially those focused on preserving and restoring genetic diversity and maintaining population connectivity across the Mediterranean.

Three new sequences of *Ostrea stentina* and the evolution of the mitogenome of the *Ostreinae clams* (Ostreidae, Bivalvia)

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1. Context and objective of the study Oysters are a significant component of marine ecosystems and have long served as a valuable food source for humans. These bivalve mollusks belong to the family Ostreidae. Identifying oyster species is often challenging due to the high plasticity of shell morphology. Individuals of the same species can look different, while those from different species may appear similar. This intra- and interspecific variation complicates species delimitation and contributes to taxonomic inflation. The use of molecular data has greatly improved both species identification and the understanding of their evolutionary relationships. In particular, mitochondrial genome-derived markers have proven essential for these purposes. In this study, we sequenced three complete mitochondrial genomes of *Ostrea stentina*, commonly known as the dwarf oyster and we conducted a mitogenomic comparative and evolutionary study, combining the new sequences with all available data for the Ostreinae.

2. Materials - methods Genomic DNA was extracted using the commercial Invisorb kit. Genomic libraries were constructed using the commercial Illumina kit. Libraries were sequenced with AVITI. Multiple alignments of the protein-coding genes were done with MAFFT and through TranslatorX. The phylogenetic analyses were conducted using IQ-TREE2. Statistical analyses were conducted considering various aspects such as codon distribution and compositional biases, both calculated with MEGA and Excel.

3. Results The mitogenome of *O. stentina* displays the typical gene order found in the subfamily Ostreinae, which differs from the arrangements observed in other Ostreidae subfamilies. Analysis of these mitogenomic arrangements identified conserved gene blocks inherited from the mitogenome of the last common ancestor of Ostreidae. Comparative analyses highlighted distinctive features of Ostreinae mitogenomes, including specific traits in protein-coding genes, tRNA and rRNA genes, as well as control regions. Mito-phylogenomic analyses indicated that the genus Ostrea is polyphyletic. The stems and loops of several tRNAs contained short DNA motifs that serve as markers for identifying individual species or groups of species. Similarly, intergenic spacers exhibit short sequences, acting as molecular signatures for particular taxa.

4. Conclusions Mitogenomes proved to be taxonomic and phylogenetic markers that play a crucial role in unraveling the evolutionary history of oysters.

A multidisciplinary approach to invasive species surveillance: combining eDNA and citizen science to monitor the invasive American mink in Sardinia

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The semiaquatic mustelid American mink (*Neogale vison*) is an invasive species in Europe. Introduced from North America in the early 1900s for fur farming, through its opportunistic feeding habits it exerts significant pressure on native wildlife, both through predation and interspecific competition for resources. In Italy, feral populations are documented in northeastern regions, Lazio and Sardinia. However, distributional data and information on the impacts on the local fauna in Sardinia are still limited, highlighting the need for targeted research. In this study, we investigate the presence of the American mink across Sardinia through a multidisciplinary approach combining environmental DNA (eDNA), camera trapping and citizen science applications. We engaged local communities across the island to gather observational data, which were subsequently validated through targeted camera trap surveys and the identification of faecal samples in the field. To increase detection probability, we are also developing a novel species-specific eDNA assay based on qPCR technology. Specifically, we designed two new sets of primers and probes targeting ˜150 bp fragments of the mitochondrial Cytochrome b gene, which we are currently validating to evaluate their amplification efficiency and confirm their specificity, particularly in discriminating *N. vison* from sympatric mustelid species. From citizen records, camera trapping and field surveys the species resulted widespread in central and northern Sardinia over the main water courses. Tests on water samples from localities of confirmed occurrence of the target species produced positive and promising results. By integrating molecular and occurrence records, we aim to develop the first detailed distribution map of the American mink in Sardinia, paving the way for informed conservation and management actions.

Intraspecific variation of dung beetles along a geographic gradient inferred from metabarcoding data

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The study of insects' spatial distribution and their intraspecific variability is crucial for effective conservation strategies. Intraspecific variability is usually studied at the population level for a limited number of species because of the scarcity of experts and the high costs for genetic analysis. In this context, metabarcoding is emerging as a time- and cost-effective genetic technique that allows the simultaneous identification of multiple taxa within the same sample. This technique has proven useful in studying insect communities in both terrestrial and aquatic environments even at an intraspecific level. Here we focused on dung beetles, a group of Coleoptera specialized in feeding on vertebrate dung and contributing significantly to nutrient cycling and soil health. In particular, we studied intraspecific variability of dung beetles in three areas spanning the Pennine, Cottian and Maritime Alps in western Italy. For each area, three valleys located near each other were chosen and for each valley 6 sites were selected at three altitudes (1000 m, 1500 m and 2000 m) and within different habitats (pasture and wood). Dung beetles were identified with both morphology and metabarcoding and results compared to evaluate the reliability of DNA identification. Amplicon sequence variants (ASV) were inferred from metabarcoding data as a proxy of intraspecific diversity. Morphological and metabarcoding identification generally agreed, with morphology providing a higher number of species in a few samples. Metabarcoding highlighted intraspecific variability in most dung beetle species, with some ASV widespread in the three studies areas while others restricted within an area or even within a valley. Our results showed that genetic identification can help infer geographic patterns of dung beetles at multiple spatial scales at both specific and intraspecific level and hold the potential to inform conservation strategies. However, differences in species richness with morphological identification probably due to undetected rare species and incomplete reference databases for taxonomic assignments emphasize the need for further refinements of metabarcoding protocols.

Tentacular barcodes: building reference libraries for Mediterranean cephalopods

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Species delimitation by traditional morphological methods is challenging in cephalopods due to their flexible bodies, changeable pigment traits, and sometimes high morphological homoplasy. Additionally, samples could be heavily damaged during collection making the correct identification of the specimens at species level more difficult. Correctly identifying the species is critical to evaluate their distribution, abundance and role in the ecosystem. This is particularly relevant for cephalopods that play crucial roles in marine ecosystems, acting as both predators and prey for apex predators, thereby contributing to the distribution of energy and nutrients across the food web.

In this context, DNA barcoding offers a useful and powerful tool for reliable and rapid taxonomic assignment. Despite the important possibilities, this approach is partially limited by the insufficient coverage of reference sequence databases and the inaccuracy in their taxonomic labels.

With the present work, we aim to produce a curated multimarker reference library for the 72 cephalopod species inhabiting the Mediterranean Sea. Moreover, analysing public sequences the robustness, reliability and completeness of the online databases (NCBI and BOLD) are evaluated. Along with new 'referenced' sequences for the Mediterranean cephalopods, our results provide clear evidence of errors in public repositories such as: a) mislabeling and/or misidentification, b) old nomenclature and/or data not updated, c) the presence of many possible new species not yet described and d) clades which require further analysis to solve taxonomic uncertainties. In particular, the obtained results suggest careful scrutiny and manual corrections of publically obtained sequences by experts prior to their use.

Overall, our results highlight the current limits in studies on cephalopods using barcoding and/or metabarcoding approaches, mainly linked to the low species coverage rate of available sequences, taxonomic uncertainties and the relative low reliability of public databases. Further work is needed in order to complete and improve the curated reference libraries, basic requirements in molecular studies.

Population genetics and local adaptation of the Mediterranean *Chamelea gallina* (Mollusca-Bivalvia) for conservation and management

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The bivalve *Chamelea gallina* (Linnaeus, 1758) represents one of the most economically significant resources for fisheries in the Mediterranean basin and in particular in Italy. The Food and Agriculture Organization of the United Nations (FAO) reports an average total annual catch of about 60,000 tons (2004-2013) in the Eastern Atlantic, Mediterranean and Black Sea. Over the last three decades, clam beds have been progressively reduced. This reduction has had consequences on fishing activity with serious implications both at social and economic level, not only for fishermen, but also for employers of the fishing industries. Despite its economic interest, knowledge about the genetics and biology of this species is rather scarce. Therefore, this study aims to define the degree of genetic variability in natural beds of *C. gallina*. The high degree of genetic variability is an indicator of the good health status of a species and confers the ability to cope with environmental disturbances. In this population genetics study, specimens from 8 selected sites located in the Mediterranean Sea, Black Sea, and Atlantic Ocean were collected and analyzed through ddRAD sequencing, providing for the first time a holistic view of the genetic variability of *C. gallina*. In addition, specimens of congeneric species *Chamelea striatula* (da Costa, 1778), collected in one site from Atlantic Ocean and one site from North Sea, were analyzed. This omics approach allowed to generate dense genetic markers that were correlated with morphological and structural features of the shell to unravel whether certain phenotypic aspects may depend on locally adapted genetic traits. The results of this study represent a valuable tool for the management of this halieutic resource and to implement, where necessary, conservation measures and restocking programs with a positive social and economic impact on Italian and Mediterranean fisheries.

Assessment of the genetic diversity of *Callinectes sapidus* reovirus 1

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The frequency of epizootics is increasing in marine ecosystems, affecting marine species and communities. One of the pathogens involved in these events is *Callinectes sapidus* reovirus 1 (CsRV1). This virus has a segmented double-stranded RNA genome consisting of 12 segments, and it is distinguished by high mutation rates, short periods of generation, and segments recombination and reassortment.

In this context, we provide phylodynamic reconstruction employing a genomic approach, utilizing genomes and segments from the NCBI virus database. Molecular dating based on the whole genome reveals a temporal origin around 40 years ago, followed by an increase in genetic variability and expansion of the viral population around 10 years ago. The estimated evolutionary rates for all 12 segments are similar, indicating that none of the segments are more subject to selective pressure. Furthermore, the neutrality test indicates that most of the segments encoding proteins with strongly conserved functions are not under selective pressure.

Until now, this virus has been detected in individuals from the American Atlantic coasts. However, since *Callinectes sapidus* is an alien species that has been present in the Mediterranean Sea for several decades, we are also investigating the potential presence of CsRV1 in samples mainly from Sardinian specimens, in order to broaden our understanding of its genetic variability and adaptive potential.

Constant genetic surveillance of CsRV1 in geographic sites where its host is present is crucial due to the threat of genetic reassortment and the potential for the emergence of more hazardous variants. This is even more important in the case of invasive species, as they have the potential to introduce unknown pathogens that could cause risks to endemic species.

Aotearoa marine biodiversity: unlocking New Zealand's Chondrichthyan fauna diversity with DNA barcoding

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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. Aotearoa New Zealand supports a remarkably diverse chondrichthyan (sharks, rays, and chimaeras) assemblage, hosting over 10% of the global chondrichthyan diversity. About 20% of species are endemic to the region. Novel species continue to be described regularly, and taxonomic uncertainties require further investigation. This knowledge gap is reflected in the fact that over one-third of the New Zealand chondrichthyans are assessed as regionally Data Deficient. 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 chondrichthyan species caught on Chatham Rise (New Zealand) during a scientific bottom trawl survey. Each specimen was analysed using COI and NADH2 mitochondrial DNA markers.

This study enhances the molecular data available on chondrichthyans by providing new NAHD2 and COI sequences for 14 putative species belonging to 12 different genera of four different orders (Chimaeriformes: *Harriotta* and *Hydrolagus*, Carcharhiniformes: *Bythaelurus*, Squaliformes: *Centrophorus*, *Centroselachus*, *Deania*, *Dalatias*, *Etmopterus*, and *Scymnodon*, Rajiformes: *Amblyraja*, *Bathyraja*, and *Dipturus*), many of which have little genetic data available.

Trophic habits of the endemic and threatened *Lampetra zanandreai* (Vladykov, 1955): first insights from the LIFE Minnow project

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Small-sized freshwater species with little or no direct economic value are often threatened yet poorly understood from an ecological perspective. *Lampetra zanandreai* (Vladykov, 1955) is a non-parasitic, strictly freshwater lamprey endemic to the ancient Po basin and threatened with extinction. Apart from some general information on its biology and life cycle, much of its ecology, including its feeding habits, remains unknown. This study aimed to investigate the feeding habits of the Po brook lamprey using a molecular approach.

Twenty-seven specimens and sediment samples were collected from two different streams in the upper Po basin (NW Italy) and analysed. The content of the digestive tracts were collected in the laboratory and preserved in absolute ethanol. Species composition of these contents was investigated through DNA metabarcoding using the primer set 1391f/EukBr targeting the V9 hypervariable region of eukaryotic 18S rDNA. Sediment samples were also analysed using the same metabarcoding protocol to identify potential trophic sources.

Taxa other than lamprey were found in 15 out of 27 individuals. The most frequently found taxon in the digestive tract contents was Ciliophora (found in 9 individuals), followed by Euglenozoa (5 individuals), Chlorophyta and Nematoda (2 individuals each), Cryptomycota and Mollusca (1 individual each). All the taxa were also found in the sediment with the exception of Mollusca.

This is the first molecular assessment of the diet of *L. zanandreai* and provides new insights into the trophic ecology of this endemic, threatened and neglected species. A more detailed analysis using additional primers is underway. Understanding the ecological requirements and needs of different species is essential for effective conservation strategies, and diet is a key ecological parameter.

Investigating biodiversity of edaphic fauna: integrative taxonomy of Italian Pauropoda (Myriapoda)

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Soil ecosystems harbour a rich biodiversity and a complex community of different animal taxa known as edaphic fauna. Among these, microarthropods play a crucial role in nutrient cycling and soil transformation. Many studies focus on investigating this biodiversity and unravelling the relationship among different taxa; unfortunately, there are some animal groups for which the species identification is harder for the lack of morphological and molecular data. One of these groups is Pauropoda (Myriapoda), microarthropods that live in the first layers of soil. Their main taxonomic features are the segmented antennae with three flagella and an antennal globulus, and the morphology of the anal plate. The "taxonomic impediment" is particularly high for Pauropoda since published data mostly derive from a single author (Ulf Sheller; now deceased), molecular data are scarce, and no dichotomous key is available to enable a reliable species identification.

This research aims to improve morphological and molecular approaches for the species identification to expand the knowledge on the diversity of Italian Pauropoda using an integrative approach.

Sampling took place in nine localities of Emilia Romagna and Tuscany. Before undergoing molecular analyses, specimens were fixed and conserved in ethanol 70°-100° at -20°C, mounted on slides with glycerol and ethanol 70°, and observed with optical microscope up to 40x for species identification and to take pictures of the main taxonomic features. In addition, some specimens were used to observe taxonomic features with Scanning Electron Microscope (SEM).

A non-destructive DNA extraction was performed to ensure the retrieving of the carcass, which was subsequently mounted on slides with solution Gisin A (lactic acid, glycerol and formalin), as voucher specimen. Ultimately, amplification of COI and 18S genes was performed to obtain DNA sequences used for taxa identification and phylogenetic analyses.

Some species were recorded for the first time in Italy, expanding their known distribution range. Also, the integration of classical taxonomy with non-destructive DNA extraction and SEM imaging offers a promising framework for species-level identification.

Further analyses and broader sampling will be essential to clarify phylogenetic relationships and improve the taxonomic resolution of this group.

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Integrative taxonomy towards NIS: the case of *Pseudodiaptomus* spp.(Copepoda, Calanoida) from the Mar Piccolo of Taranto (Ionian Sea)

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Pseudodiaptomus marinus (*Pseudodiaptomidae*, Calanoida, Copepoda) is a demersal species found for the first time in Japan in 1913 and in many other parts of the coastal seas around the world. About 100 years later (in 2009), it has been reported for the first time in the Mediterranean Sea from Italy, in the north Adriatic Sea. Since that date this species has been repeatedly reported as an invading strategist in at least 13 other sites in all the sectors of the Italian Seas and identified with both morphology and DNA. The rapid spreading of this species interested also the Mar Piccolo of Taranto, in the southern Italy, and stimulated the creation of a study group to better monitor its fast diffusion. In 2021 an in-depth observation of the morphology of a couple of *Pseudodiaptomus* adults suggested the possible presence of a co-generic, different species. During 2024, a dedicated sampling effort (close to the sea bottom) was carried out to collect additional *Pseudodiaptomus* individuals from the Mar Piccolo. Among the specimens collected, two morphologies were clearly distinguishable: one ascribable to *P. marinus* and another one with marked differences (as total body size, female genital segment, and male P5). Molecular analysis, involving the COI mitochondrial marker, revealed no match with sequences already present in GenBank and ascribed to *P. marinus*. The newly obtained sequences resulted close to *P. hessei* (18% of COI homology) and to *Labidocera rotunda* (20% of COI homology), confirming the Mar Piccolo specimens belong to a different and probably still not known species. Future analysis including specimens belonging to the morphologically more similar congeneric species, i.e. *P. trihamatus* (native to the Philippines), are needed to finally assess the species identity of these interesting specimens and to clarify the Pseudodiaptomus systematics. The co-existence of co-generic species in the same habitat, also rises problems of competition and/or high adaptability of species in coastal brackish inlets.

Uncovering Mediterranean mesophotic corals through integrative taxonomy

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Mesophotic coral ecosystems (MCEs), occurring at a depth range of approximately 30-200 meters, are recognised as biodiversity hotspots and potential refugia for shallow-water species, increasingly threatened by anthropogenic pressures. Despite their ecological importance, MCEs remain globally vulnerable, partly due to a limited understanding of their species diversity and distribution. Accurate species identification is crucial to implement conservation strategies; however, traditional coral taxonomy, based mainly on corallite morphology, is challenging and prone to ambiguous results. In this context, integrating traditional morphological approaches with modern molecular tools offers a more reliable framework for species identification.

This study aims to implement an integrative taxonomic approach to investigate the coral assemblage of a recently discovered mesophotic coral reef off the Apulian coasts (southern Italy), mainly built by two scleractinians, *Phyllangia americana mouchezii* (Lacaze-Duthiers, 1897) and *Polycyathus muellerae* (Abel, 1959). The latter, together with other scleractinians and bioconstructor invertebrates, built a structurally complex substrate that hosts numerous associated species. Twenty-three scleractinian colonies were sampled and after their morphological identification a subsample from each colony was collected and processed for further molecular analyses. Genetic diversity was assessed using both mitochondrial (i.e. COI) and nuclear (i.e. ITS2 and 28S) markers.

Morphological and molecular analyses were largely consistent except for two samples, one identified morphologically as *P. muellerae* but molecularly as *P. a. mouchezii*, and the other vice versa. However, these latter species belong to the same family Caryophylliidae and may eventually show partially overlapping morphologies both in the shape of the colony and of the individual corallites, making it difficult to determine whether subtle variations in morphological characters are intra- or interspecific. By providing reference genetic data for the unambiguous identification of Mediterranean scleractinians, this study proved the effectiveness of an integrative approach in resolving species boundaries within complex coral assemblages, paving the way for future taxonomic, ecological, and conservation-oriented research in these fragile mesophotic ecosystems.

Genetic insights into *Salmo letnica* morphotypes in Lake Ohrid

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Salmo letnica, commonly known as the Ohrid trout, is an endemic species inhabiting Lake Ohrid, a deep and ancient lake situated on the border between Albania and North Macedonia. Within this species, four distinct morphotypes, *S. l. typicus*, *S. l. aestivalis*, *S. l. balcanicus*, and *S. l. lumi*, have been described based on differences in morphology and reproductive behaviour. However, comprehensive genetic data are limited, making it difficult to determine the degree of differentiation among these morphological forms and to clarify their evolutionary relationships. In this context, this study aimed to explore the genetic diversity and population structure of the four morphotypes using the mitochondrial Control Region as a molecular marker. Samples, representing the four *S. letnica* morphological forms, were collected from various locations along the Albanian shoreline of Lake Ohrid. A total of 127 sequences (553 bp) were obtained and compared with those from other *Salmo* species. Results from phylogenetic and clustering analyses indicated a reduced genetic differentiation among the four morphotypes, suggesting that their divergence may be primarily ecological and not yet reflected in mitochondrial DNA. In contrast, a phylogenetic analysis including all *Salmo* species reported from Lake Ohrid evidenced a clear genetic separation between *S. letnica* and *S. ohridanus.* This outcome is consistent with previous studies and reinforces the genetic divergence of *S. ohridanus* from its closely related species. Additionally, the findings supported the hypothesis that *S. letnica* evolved within Lake Ohrid from ancestral *S. farioides* populations, with local environmental factors driving their differentiation. Further research, including broader sampling and additional molecular markers for both *S. letnica* and *S. farioides*, is needed to better understand the evolutionary and ecological dynamics shaping these trout morphotypes.

Tracking the overlooked *Didemnum pseudovexillum* in the Mediterranean Sea: insights from integrated taxonomy

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Didemnum pseudovexillum Turon & Viard, 2020 is a recently described colonial ascidian, often misidentified as the morphologically similar *D. vexillum.* The latter is recognized as a fast-growing species with a high potential for invasiveness, posing a threat to native biodiversity and creating challenges for the maintenance of maritime infrastructure as well as for shellfish aquaculture operations. Due to their cryptic resemblance of *D. vexillum* and *D. pseudovexillum*, the true distribution of the latter in the Mediterranean Sea remains largely underestimated. This study aimed to investigate its occurrence in two major hotspots for non-indigenous species introduction along Italian coasts: the Venice Lagoon and the Gulf of Taranto.

Colonies were manually collected from anthropogenic substrates, including piers and aquaculture structures, and subjected to detailed morphological analyses and DNA barcoding of the mitochondrial Cytochrome Oxidase I gene. Key morphological traits such as spicule size and ray number, gonad morphology, and larval structures were documented to differentiate D. pseudovexillum from its co-generic species.

Our findings confirmed the presence of *D. pseudovexillum* in both locations, with colonies showing consistent diagnostic morphological features. These identifications were further validated through DNA barcoding and phylogenetic analyses. Colonies were observed during multiple seasonal surveys but were predominantly found in the autumn period in which also larvae were found.

These results provide new records of D. *pseudovexillum* in the Mediterranean and highlight its capacity to thrive on artificial substrates, indicating its important but often overlooked role in biofouling communities. *Didemnum pseudovexillum* ability to colonize a variety of man-made structures suggests a potential for invasiveness, which warrants close attention in future monitoring efforts. The study underlines the need for integrated morphological and molecular approaches in tracking cryptic non-indigenous species and provides essential baseline data to inform surveillance and management strategies aimed at mitigating ecological risks.

Watching the wildcat: camera traps and non-invasive surveys in Sardinia

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The wildcat population living in Sardinia (*Felis silvestris lybica*) was introduced from Northern Africa into the island during Neolithic or Iron Age. Despite its long history on the island and potential evolutionary separation from other wildcat groups, information about this taxon remains limited. Sardinian wildcats are threatened by habitat fragmentation, illegal or incidental killings and potential hybridization with free-ranging domestic cats. Classified as Least Concern by the IUCN Red List due to its broad distribution across North Africa, the species *F. s. lybica* may still face local threats, particularly in insular or fragmented populations. Within this project, we applied an integrated approach of non-invasive surveillance techniques to investigate the current distribution of the Sardinian wildcat, all over the regional territory where its presence has been documented. Camera-trapping has been employed in various sites combined with the use of attractants - such as catnip in different forms - on wooden sticks, with the aim of collecting non-invasive samples available for genetic analyses. Initial findings from field monitoring and non-invasive sampling are presented in a preliminary map outlining the species' distribution, with wildcat presence registered in 40 cells when using a 10 x 10 km quadrant grid over the island. Further research will allow to shed light on this elusive species and enhance its conservation.

Morphological and molecular characterisation of Italian *Leptoconops* species with new recorded populations.

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The genus *Leptoconops* is one of the 4 blood-feeding genera of midges within the family Ceratopogonidae (Diptera). Although studies have been conducted on this genus over the past century, uncertainties and ambiguities persist regarding the identification and classification of many species. Molecular data remain scarce, and nothing is currently known about the potential vectorial role of these midges, despite their well-documented nuisance and painful bites, which can cause allergic reactions. These considerations are particularly relevant to the study of *Leptoconops* in Italy, where 6 species are currently known. Research has so far been limited to the Maremma area (Tuscany), characterized by coastal marshes and sandy shores, habitats that support all life stages of *Leptoconops*. Our study aimed to provide new morphological and molecular data to resolve identification issues, explore the presence of *Leptoconops* in previously unstudied areas, and implement a suitable methodology for pathogen detection in this genus. During the summer of 2024, we collected over 4,000 adult midges using traps and aspirators across two districts: the coastal area of Grosseto (Maremma) and the inland hilly district of Siena. Following morphological analyses, DNA was extracted from representative individuals for DNA barcoding, and genome sequencing was performed on two species: *L. noei* and *L. irritans*. A new qPCR protocol for pathogen detection was developed using a high-throughput microfluidic platform. Morphological identification enabled the assignment of specimens to 4 species, each showing distinct geographical distribution. Notably, the collection of *L. noei* specimens from 12 sites in the Siena district represents the first recorded presence of *Leptoconops* in an Italian inland area. SEM analyses revealed previously undescribed morphological traits and variability in sensory organs. Detailed morphological observations, combined with new DNA barcoding analyses, clarified longstanding ambiguities in the taxonomy of *Leptoconops* species. Genome sequencing is ongoing, and we have successfully annotated the mitochondrial genomes of both *L. noei* and *L. irritans*. The pathogen detection protocol has been optimized, and screening is currently underway. Our research has significantly expanded the knowledge of the distribution, morphology, and genetics of the genus *Leptoconops*, while also introducing novel tools for species identification and pathogen detection.

In-Deep conservation: using genetic tools to protect a rare and threatened deep-sea shark

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Sharks of the genus *Oxynotus* (Chondrichthyes: Oxynotidae) are rare and poorly known benthic elasmobranchs, distributed in deep marine environments worldwide. Among them, the angular roughshark *Oxynotus centrina* (Linnaeus, 1758) is the only species of the genus reported in the Mediterranean Sea. Due to its biological traits, it is highly vulnerable to fishing pressures. Moreover, having experienced in the recent past a severe population decline in this area, it has been listed as Critically Endangered in the IUCN Red List.

Through the analysis of two mitochondrial markers (COI and NADH2) widely used in elasmobranch studies, this work aims to investigate the intraspecific diversity and population connectivity of the species. Furthermore, the newly produced sequences were used to investigate the phylogenetic relationships among the species of the genus *Oxynotus.* To reach this scope, a total of 80 tissue samples were collected in Sardinian waters and from other Mediterranean areas, and the sequences newly produced here were compared with those already available in online databases.

Preliminary results based on the concatenated dataset (COI+NADH2) indicate very low levels of genetic variability and a low number of haplotypes in *O. centrina*, suggesting a potential high connectivity among areas. Phylogenetic trees show a close relationship between the sailfin roughshark *O. paradoxus* Frade, 1929 and the prickly dogfish *O. bruniensis* (Ogilby, 1893), clearly separated from *O. centrina.* In addition, COI sequences suggest the occurrence of possible cryptic species and mislabelling/misidentification within the genus.

Our results provide new genetic data for this poorly known species and lay the basis for future research that should encompass all the species included in the genus, presently lacking molecular characterization. Given the vulnerability of deep-sea species to environmental changes and human impacts, the implementation of baseline information is awaited to be provided for additional species, as it is essential for developing informed management and protection plans.

Avian flu in marine mammals: a phylodynamic and prevalence analysis

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The issue of avian influenza is becoming increasingly significant on a global scale. In recent years, the virus has been responsible for the death of thousands of marine mammals, underscoring the need to thoroughly study its behaviour in these species. Over the course of its evolution, the virus has demonstrated a remarkable ability to adapt to new hosts, successfully crossing species barriers multiple times. In this study, we performed a phylodynamic reconstruction of all strains isolated in marine mammals, with the aim of identifying the expansion capacity of different lineages and estimating the speed at which they are transmitted from one host to another. Additionally, we conducted a prevalence analysis of all isolates available in databases to determine whether prevalence varies between hosts depending on the clade. Should significant differences be identified, we aimed to investigate whether such variations are linked to specific genomic mutations capable of influencing the fitness of the strains involved. Our analysis on all available genomes revealed a clear structuring concerning the different clades, whereas no significant genetic structuring was observed with regard to the hosts. No sites under selective pressure have been detected. Genetic variability, in fact, seems to increase in relation to different geographical areas, while still maintaining avian hosts as the primary and preferred hosts. Notably, marine mammals exhibit a pattern markedly different from that of non marine-mammals. The fact that their pattern is more similar to birds rather than dairy cattle suggests that there has been no substantial adaptation to the new host. It is plausible to hypothesize that marine mammals were exposed to the virus through the consumption of carcasses of infected birds. In the case of adaptation, hemagglutinin at the binding site would exhibit specific mutations to effectively bind to sialic acid present on host cells, increasing the virus's fitness. This mechanism was clearly observed in the case of the H1N1 virus, which showed an adaptation that allowed it to rapidly spread among the human population, leading to a pandemic. This study not only elucidates the molecular dynamics of viral spillover but also provides critical insights into how cross-species transmissions can affect animal population dynamics and interspecific interactions within diverse ecosystems, and our findings contribute to a broader understanding of wildlife health.

Molecular monitoring of the invasive species *Aleurocanthus spiniferus* (Hemiptera: Aleyrodidae) for rapid identification and invasive route reconstruction

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The recent spread of invasive insect species represents a growing threat to agricultural systems across Europe. Among them, the polyphagous orange spiny whitefly ("OSW"; Aleurocanthus spiniferus) has caused significant economic losses in different regions of Italy, severely affecting crop production and increasing management costs. Given its harmfulness, OSW has been classified as a quarantine pest of concern to the European Union. Moreover, while associated alien parasitoids could contribute to this species' biological control, their introduction may pose risks to native biodiversity. This project, in collaboration with the Emilia-Romagna Regional Plant Protection Service, aims to assess genetic diversity and investigate invasion routes for OSW in order to develop effective control strategies. In parallel, a study on the identification of exotic associated parasitoids is carried out.

Specimens of *A. spiniferus* were investigated in the Emilia-Romagna region, in particular in Modena province, using an integrative approach combining morphological identification with molecular analyses. 1,800 leaves of *Malus domestica*, *Pyrus communis* and *Vitis vinifera* were sampled to evaluate OSW infestation. DNA was extracted from field-collected individuals, and mitochondrial COI and rRNA 16S genes were amplified using species-specific primers to confirm species identity and genetic diversity.

The results revealed marked differences in the abundance of *A. spiniferus* puparia, with *V. vinifera* showing the highest levels of infestation. Genetic analyses revealed the presence of three distinct haplotypes of OSW – including one reported for the first time in Europe, previously found only in China. Additionally, parasitoids emerged from OSW specimens and were identified as Encarsia nipponica, an exotic species originating from Asia – the first record for this species in Europe.

These findings provide novel genetic data on *A. spiniferus*' populations in Italy and offer new insights into its distribution patterns and invasion dynamics. The detection of exotic parasitoids, such as *E. nipponica*, highlights the potential for future studies on OSW-associated parasitoids. Further investigations will expand the analysis to other infested areas and contribute to risk assessment and pest management planning at regional level.

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Molecular phylogenetic position of *Setopus* (Gastrotricha, Chaetonotida)

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Gastrotricha is a phylum of microscopic aquatic animals that includes over 900 species, with approximately 520 belonging to the order Chaetonotida. The internal phylogeny of this order remains unresolved, as many genera and families have been found to be non-monophyletic. This is especially true for the group Oiorpata, which comprises the marine and freshwater benthic family Chaetonotidae, as well as the freshwater planktonic families Dasydytidae and Neogosseidae. The lack of a robust phylogenetic hypothesis to explain the relationships within this group arises primarily from a limited amount of molecular data, which is often missing for entire genera. One notable example is the rare genus *Setopus*, which consists of nine described species that share a planktonic lifestyle and the morphological features associated with this ecological adaptation. Our study aims to develop a phylogenetic hypothesis based on the first available molecular data for *Setopus* to clarify the relationships of this genus within the Oiorpata clade. Specimens of an undescribed species (*Setopus* n. sp.) and *S. tongiorgii* were collected during sampling campaigns in various regions of Italy. Identification was conducted using Nomarski microscopy, while molecular data were obtained through a bioinformatics pipeline utilizing Whole Genome Amplification and Whole Genome Sequencing. The resulting reads were analysed to obtain sequences for three genes: 18S rDNA, 28S rDNA, and mtCOI. The new dataset was combined with published data from over 100 selected chaetonotidan species, ensuring that at least one set of sequences from each of the currently accepted genus was included where available. A preliminary phylogenetic analysis shows the two Setopus species clustering together among other planktonic species, with the indication of Ornamentula being its sister taxon. Surprisingly, Dasydytidae appears paraphyletic due to the nested position of species currently included in Chaetonotidae. If confirmed by the ongoing analyses, these findings may significantly contribute to understanding evolutionary relationships within the Oiorpata lineage.

Detecting *Callinectes sapidus* expansion through eDNA: a case study from inland waters of Friuli Venezia Giulia

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Callinectes sapidus (Rathbun, 1896), native to the western Atlantic Ocean and the Gulf of Mexico, has become a prominent invasive species in the Mediterranean over the past decade. Its success is largely attributed to its high adaptability and aggressive predatory behaviour, which have led to its classification as a highly invasive species. Since the early 20th century, it has spread into the Mediterranean Sea, raising concerns about its potential ecological and economic impacts.

The blue crab thrives in both marine and brackish environments, with its life cycle closely tied to variations in salinity and temperature. Juveniles and adults typically inhabit estuarine and coastal waters, where salinity levels fluctuate. For reproduction, females migrate to high-salinity areas to spawn, and larvae develop in these saltier environments before dispersing into lower-salinity estuaries.

In this context, the ANNOTATE project monitors *C. sapidus* through environmental DNA (eDNA) in river tributaries flowing into the Marano and Grado Lagoon (16,364 ha, Northern Adriatic). Although the species has been established in the lagoon since the early 2000s, this study investigates its ongoing expansion into connected freshwater environments, with sampling conducted at 48 sites.

Surface water from six rivers was sampled monthly during summer and fall at two sites per river. Water parameters were recorded, including temperature, salinity, conductivity, dissolved oxygen, and pH. eDNA was extracted from the water samples and analyzed using a species-specific COI barcoding assay based on hydrolysis probe chemistry in qPCR to detect the presence/absence of *C. sapidus.*

Initial results revealed considerable variation in temperature and salinity across both rivers and sampling points. Mean temperature ranged from 17.7°C to 21.5°C, while salinity values varied from 3.5×10 ppm to 238 ppm. *C. sapidus* was detected in all surveyed tributaries, including the northernmost sampling points, located approximately 20km from the river mouths.

Overall, these findings confirm the species' capacity to tolerate a broad range of environmental conditions supporting its continued ecological expansion. Moreover, this study highlighted the effectiveness of eDNA as a powerful tool for detecting the distribution of invasive species in aquatic ecosystems.

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Safeguarding a freshwater sentinel: eDNA-Based conservation of *Austropotamobius pallipes* across borders

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Autochthonous crayfish populations have been declining in the past years due to water pollution, climate change, invasive species, and pathogens. In this context, project PALLIPES (Interreg Italia-Slovenija, ITA-SI0600154) aims to conserve and enhance the population and environment of *Austropotamobius pallipes* in the transboundary area between Italy and Slovenia. This species, listed as endangered in the IUCN Red List, is protected by the Habitat Directive and the Bern Convention. It is a biological indicator for freshwater ecosystems, hence the necessity to assess its population and the state of the environment. An established tool for the monitoring of rare, cryptic, and invasive species is environmental DNA (eDNA). This method allows for tracing species through their genetic material that can be detected in the environment. It is also efficient at uncovering small local populations that might have been neglected by traditional methods. Water samples (1.5L) were collected in the river Reka-Timavo at 120 different Slovenian sites between June and October 2024. Water was filtered using a peristaltic pump onto Sterivex 0.45m filters and then conserved until eDNA extraction. qPCR was performed on a species-specific assay based on hydrolysis probe, specific for *A. pallipes*. This step was also executed with probes specific to *Procambarus clarkii* (invasive crayfish) and *Aphanomyces astaci* (pathogen) to verify their presence/ absence in the sites where *A. pallipes* was not detected. Barcoding analysis revealed the presence of *A. pallipes* at 42 sites and *P. clarkii* at 9 sites. No sites tested positive for *A. astaci*, suggesting that observed declines in *A. pallipes* populations are more likely attributable to environmental stressors, such as water quality degradation and climate change, rather than to crayfish plague. Both *P. clarkii* and *A. astaci* are currently under further investigation as they have been detected in the past, and we cannot exclude their influence on the local populations yet. Moreover, *P. clarkii* eDNA could originate from domestic wastewaters linked to human consumption, so the presence evaluation is still underway. To conclude, *A. pallipes* is still found in the Slovenian territory, thus, conservation measures must be implemented to preserve the local population. The same methodology will be applied for the Italian territory for the creation of a shared strategy for conserving *A. pallipes* within the transboundary area.

Toward photoablation of larval salivary glands in the tiger mosquito *Aedes albopictus*

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Mosquito larval salivary glands (lSGs) remain poorly characterized, despite the key role in pathogen transmission of adult salivary glands. Since larval stages represent a critical window for mosquito development and vector control, we aimed to develop a functional tool to manipulate lSGs in vivo, building on our previous morphological and molecular characterization of *Aedes albopictus* lSGs.

Morphological analyses, using transmission electron microscopy (TEM) and histological staining, combined with lipidomic and proteomic profiling, revealed a dense vesicle network, intense secretory activity, and a high lipid content. To further investigate lSGs function, we employed a targeted cell photoablation strategy using miniSOG2. MiniSOG2 (mini Singlet Oxygen Generator 2) is a green fluorescent protein that, upon blue-light illumination, produces singlet oxygen (1O), inducing localized oxidative damage. A salivary gland–specific promoter, identified based on previous studies, was cloned upstream of miniSOG2 coding sequence and inserted into a PiggyBac transposon vector system.

Ultrastructural and histological analyses revealed densely packed secretory vesicles and glandular organization consistent with active secretion. Staining assays highlighted a high lipid content within the lSGs, which was further corroborated by proteomic profiling, identifying proteins involved in lipid metabolism. Lipidomic data also supported these findings, showing enrichment in lipid species as glycerolipids. Microinjection of PiggyBac constructs into *Aedes albopictus* eggs led to stable integration of the transgene. The presence of miniSOG2 transcript in larvae was confirmed by PCR on cDNA, validating activity under the selected salivary-specific promoter. Upon light stimulation, targeted photoablation of the salivary tissue is planned to assess the construct and to explore the physiological role of larval salivary glands.

Understanding the specific functions of lSGs could reveal whether they play roles in feeding, osmoregulation or immunity, which remain largely speculative at this stage. Overall, this work offers new insights into *Ae. albopictus* physiology and could support future efforts to control mosquitoes at early developmental stages.

Sessione
Nuove frontiere della Zoologia: innovazione e sinergie interdisciplinari

Comunicazoni

Ecohydraulics meets Zoology: a powerful partnership for river habitat assessment and modeling

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As environmental challenges intensify, the integration of ecohydraulics and zoology is emerging as a critical frontier for advancing river habitat assessment and modeling. This talk explores the powerful synergy between these disciplines, with a particular focus on the crucial role that species-specific biological knowledge plays in interpreting eco-hydraulic model outputs and predicting ecological responses.

Understanding the biology of aquatic and semi-aquatic species, such as their flow preferences, swimming capabilities, reproductive strategies, habitat use across life stages, and sensitivity to different environmental variables, is fundamental for linking hydraulic and geomorphic conditions to ecological relevance. Without this ecological context, hydrological and geomorphological outputs (e.g., velocity, depth, shear stress, sediment size, channel geometry) remain just physical quantities. However, when interpreted through the lens of species biology, they become indicators of aquatic habitat suitability, enabling us to translate physical metrics into meaningful ecological predictions.

In this framework, species traits guide the selection of relevant hydraulic and geomorphological parameters and help define threshold values for habitat availability in rivers. These biological requirements inform both the structure of habitat models and the interpretation of spatial and temporal patterns derived from hydraulic simulations across scales.

We present recent advances in high-resolution hydrodynamic modeling, species distribution modeling, and trait-based ecological frameworks that enable the estimation of species presence probability as a function of flow dynamics and river geomorphological characteristics. By combining ecological insight with robust physical modeling, this interdisciplinary approach enhances our ability to assess habitat quality, forecast species responses to hydrological changes, and design effective conservation strategies.

Through case studies, this talk will illustrate the current capabilities and future potential of ecohydraulics-zoology integration for guiding habitat restoration, environmental flow management, and biodiversity conservation under increasing anthropogenic and climatic pressures. Emphasizing collaborative approaches and knowledge exchange between engineers and zoologists, we aim to demonstrate how species biology is not just a complementary layer, but a foundational component for meaningful river habitat modeling.

Interdisciplinary insights into human–wildlife interactions: spatial responses of wolves and roe deer to anthropogenic pressures

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Human activities are increasingly reshaping wildlife behavior and spatial ecology, particularly for large mammals that require extensive, connected habitats. Understanding how multiple anthropogenic pressures influence species distributions and spatial behavior is critical for conservation planning. This study focuses on the spatial responses of wolves (*Canis lupus*) and roe deer (*Capreolus capreolus*) to various human activities, including livestock grazing, hunting, recreational activities, urbanization, infrastructure development, and landscape fragmentation. Adopting an interdisciplinary and innovative approach, we integrated camera trap data, GPS collar tracking, and genetic analyses in the western Alps to understand ecosystems processes. Spatial models were used to assess co-occurrences, habitat selection and movement patterns in relation to human disturbance. Our results reveal complex spatial responses: roe deer exhibited increased selection of high-wolf-density areas during the hunting period, supporting the risk enhancement hypothesis, where avoiding one predator increases exposure to another, and highlighting the temporary impact of hunting on predator–prey dynamics. During the period of the wild boar drive hunt, roe deer also showed stronger selection for proximity to buildings, supporting the human shield hypothesis. We also modelled the co-occurrence probabilities of roe deer, red deer and wolves, providing important evidence of interspecific dependence, revealing that pairwise interactions among species had a greater impact than only considering environmental effects. This work demonstrates how integrated ecological methods and cross-disciplinary tools can reveal patterns of wildlife response to overlapping anthropogenic pressures. The combination of spatial, behavioral, and genetic data allowed us to disentangle cumulative impacts on mammal distribution. Our findings stress the need for conservation strategies that account for the interactive effects of human activities, and highlight the value of interdisciplinary frameworks to support adaptive, context-specific management in human-modified landscapes.

Ground-nesting bees and wasps as possible indicators of soil traits: hardness and texture

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Soil is a main habitat of terrestrial ecosystems known to harbour a rich and diverse fauna, with many groups largely recognized as relevant for soil functioning and as important indicators of soil quality. Among hymenopteran insects, ants are well-known as soil engineers, modifying soil physical structure and in influencing soil fertility through nest digging. Along other abundant edaphic taxa such as mites, collembolans and nematodes, ants can be then used as indicators of soil quality. On the other hand, ground-nesting bees and wasps (Apoidea) were almost neglected as soil modifiers, and essentially we know nothing about their potential role as soil improvers in terms of, e.g. fertility. One first step to propose ground-nesting apoids as potential indicators of soil quality is to test if different lineages of such insects are actually specialized for nesting in particular soils. Here, using data on around 70 bee and wasp species, we found that hardness and texture of nesting soils were variable among species. At species-level, soil traits seemed to vary to minor extent, but this extent also changed among species. However, interesting, certain lineages seemed to nest in a relatively homogeneous soil, and such pattern seemed to be associated with the social behaviour of the species within the lineages. In particular, accounting for common ancestry, social species nest in harder soils than solitary species. Furthermore, social species nest in soils with less % of sand and higher % of silt compared with solitary species. The texture profiles of the analysed species, when represented in a classical soil triangle classification, showed that most species fall in the spaces "sandy loam", loamy sand" and "sand", with some species, especially from social lineages, falling in the spaces "clay loam" and "silt loam". Through the intersection between pedology and behavioural ecology, our results preliminarily suggest that ground-nesting apoid communities over large areas may give insights on the distribution of soil types in the areas, and that the social behaviour of single species at a given restricted nesting area may reasonably give indirect information on soil type. Further studies devoted to the comparative analysis of other soil properties, such as organic matter content, respiration and acidity in and out of the nesting sites will help proposing ground-nesting bees and wasps as efficient indicator of soil quality.

Comparing chromatin accessibility profiles in two marine animals, the japanese clam and the european sea bass

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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 to annotate 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 PCA 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.

Investigating how global climate change impacts mosquito interaction with viruses

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Mosquito-borne diseases are re-emerging in Europe, mostly due to the establishment of new invasive vectors, in primis *Aedes albopictus*, which can transmit several arboviruses and the heart-worm. Being ectotherms, mosquitoes depend on environmental temperature to modulate their physiology, fitness, seasonal activity, and, ultimately, their interaction with viruses. Global climate change has already been shown to impact mosquito distribution and its phenology, but whether it also alters mosquito interaction with viruses is poorly understood. Global climate change manifests both as global warming, that is constant increase in environmental temperature and as increase in frequency of extreme whether events like heat waves. Global warming is a long-term challenge whereas heatwaves are temporary, short-term stress events.

We mimicked heatwaves and global warming by exposing *Ae. albopictus* mosquitoes to 32°C, a temperature above the optimum for this species, for one or ten generations generating warm-acclimated vs warm-evolved mosquitoes, respectively. We infected these groups of mosquitoes with the Cell fusing agent virus (CFAV) and studied the fitness and the response of mosquitoes to viral infection.

We saw that warm-acclimated mosquitoes fight CFAV infection, whereas mosquitoes conditioned to heat through several generations are healthier than those not exposed to a thermal challenge and tolerate CFAV infection.

These results highlight important shifts between resistance and tolerance dependent on the length of the thermal challenge. This immunological shift has far reaching implications for understanding viral transmission dynamics and has also long-term impacts on the evolution of both vectors and viruses under global climate change

Forensic zoology: The importance of an integrated zoological approach in legal cases in Italy

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In recent years, there has been an increasing use of naturalistic disciplines, including zoology, within the legal context, both during investigations and court proceedings.

This situation is well established in Anglo-Saxon countries but has been slow to develop in Mediterranean countries, including Italy. However, it is important to highlight that there is a growing attention from investigators, especially towards forensic entomology. The Italian research community is also moving very slowly in this direction.

This contribution aims to illustrate the current state of the art of forensic zoology in Italy by considering both scientific publications and forensic cases of national interest.

The work is structured into an initial presentation of articles published in recent years by Italian researchers concerning studies related to: 1) the development of methods to detect food frauds in laboratories and in the field (eg: market); 2) the evaluation of cadaver alterations caused by animals; and 3) advances in forensic entomology. In this last topic the effect of globalization and global warming on the italian entomofauna of forensic interest is also presented and discussed.

In the second part of the paper, some forensic cases that occurred within the national territory will be presented, in which zoologists were involved to provide scientific answers to investigators' questions. Additionally, some current limitations and challenges encountered in applying zoological knowledge in courtrooms will be discussed.

New frontiers in evolutionary analysis: integrating across phylogenetic comparative approaches to explore speciation and phenotypic evolution in a hyperdiverse fish clade

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Understanding how and how fast biological diversity arises is a central quest of evolutionary biology. While classical theories suggest that the rates of species origination and of phenotypic evolution should be correlated, empirical evidence to date has been mixed. Here, we investigate this relationship in wrasses, a hyperdiverse clade of marine fish. To tackle this complex question, we employ an integrated array of cutting-edge phylogenetic, morphometric, and statistical approaches. This interdisciplinary framework enables deeper insights into macroevolutionary dynamics and their potential links to microevolutionary processes.

We first constructed a novel time-calibrated phylogeny of 380 wrasse species. We captured phenotypic variation using advanced geometric morphometrics of body shape. We estimated rates of speciation and phenotypic evolution using a suite of maximum likelihood and Bayesian methods, including both tree-wide models and those allowing for localized shifts in evolutionary rates and tested for the association between rates using robust phylogenetic regression.

We found substantial variation in both speciation and phenotypic evolution rates across the wrasse phylogeny, superimposed on simpler patterns of tree-wide variation. Specifically, while speciation showed clear time-dependent variation, phenotypic evolution did not. Importantly, our integrative approach reveals how complex patterns of local variation in rates are superimposed on these general tree-wide patterns. Notably, we do find an association between rates of speciation and phenotypic evolution only for body elongation.

Our findings reveal intricate patterns of evolutionary rate variation and how the relationship between rates is complex and often trait-specific. Notably, elongation has been consistently linked to microevolutionary ecological divergence between benthic and limnetic forms in multiple teleost clades. Our results highlight the potential role of specific axes of morphological change in driving both species richness and the pace of the evolutionary process. These results underscore the importance of considering multiple phenotypic traits and lineage-specific dynamics and how simple, general, models might be largely inadequate to characterise the evolutionary history of hyperdiverse clades.

Applying deep learning and machine learning to decode animal communication signals

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In recent years, anyone interested in animal communication has wondered whether we can use machine learning and artificial intelligence (AI) to analyse communication signals and gain new information about how animals communicate. We will present two AI-powered animal communication analysis case studies, including bioacoustics and facial movement data. The applications on the datasets being proposed aim to classify signals of calls and facial configurations across a range of species. In the first task, using as models the vocal repertoires of nine Strepsirrhine primates (*Eulemur coronatus*, *E. macaco*, *E. flavifrons*, *E. fulvus*, *E. mongoz*, *E. rubriventer*, *Varecia variegata*, *Propithecus diadema*, *Indri indri*), we tested three machine learning (ML) approaches (Multi-layer Perceptrons, Support Vector Machines, and Random Forests) to compare performances in the classification of vocal types, previously submitted to feature extraction by one-third octave band filters. Despite differences across the species, we found that Random Forests performed better on average (also exceeding 80% of correct classification rate) than the other approaches. We then applied the same three methods over distances between key points estimated after a deep learning process of marker-less estimation. Our task was to identify faces where individuals from four primate species (*Propithecus diadema*, *Indri indri*, *Nomascus gabriellae*, *Saguinus oedipus*) showed an open or closed mouth. Again, we found that Random Forests performed better on average than the other approaches. We discuss how ML and AI may serve zoological research in the future by showing how flexible we can integrate feature extraction pipelines and sophisticated classification analysis on diverse communication signals.

OctoPartenopin and ink extract: novel anti-inflammatory and antioxidant from cephalopods

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OctoPartenopin is a novel antimicrobial peptide characterized from the suckers of *Octopus vulgaris*. In previous studies this peptide exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Preliminary experiments demonstrated that one of its analogues was particularly effective at both inhibiting and eradicating biofilms formed by the tested microorganisms.

In this study, we analysed the anti-inflammatory activity of OctoPartenopin using an in vitro cell culture model with Caco-2 cell culture as model. Cell cultures were treated with lipopolysaccharides to induce inflammation, and subsequently with OctoPartenopin analogues to evaluate their effects. After treatment, RNA was extracted from the cells, and differential gene expression was analyzed for markers related to anti-inflammatory and antioxidant activity.

Additionally, we explored the anti-inflammatory and antioxidant activity of extracts from ink sacs of the Sepia officinalis for similar bioactivities.

The presence of OctoPartenopin in arm suckers also provides insights into octopus behavior, particularly in relation to maternal care of fertilized eggs. These findings suggest that octopus suckers are a rich source of multifunctional peptides, with potential for the development of alternative antimicrobial agents and natural food preservatives.

Peptides derived from natural sources—such as marine organisms, amphibians, or insects—may reveal unique mechanisms of action that expand our therapeutic toolkit. Investigating these molecules not only contributes to drug discovery but also enhances our understanding of the molecular basis of inflammation, with potential applications in the treatment of chronic conditions such as arthritis, inflammatory bowel disease, and neuroinflammation.

From a gene to a material: unraveling the mechanisms of silk production in the webspinner *Embia thyrrenica*

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Human societies have long benefited from nature's resources, obtaining materials that have transformed cultures and economies. Among these, lepidopteran silk has emerged as a cornerstone of the global textile industry. In recent years, scientific attention has increasingly focused on spider silk, whose remarkable mechanical properties offer exciting potential. Silk-based materials have found new applications in regenerative medicine, biomaterials development, and drug delivery. However, multiple animal lineages independently evolved silk-like materials, and most of these represent largely uncharted territory - and an exciting avenue for novel bioinspired materials. One such underexplored group is the webspinners (order Embioptera), insects that produce silk from specialized glands of their enlarged basitarsomeres in their forelegs. The molecular mechanisms and evolutionary origins of embiopteran silk remain largely overlooked. Here, we present an integrative and interdisciplinary study of silk production in *Embia thyrrenica* Stefani, 1953 a Mediterranean endemic. We first established laboratory colonies of *E. thyrrenica* and generated a high-quality reference genome using long-reads and long-range technologies, then mapped the ultrastructure of the enlarged basitarsomeres by nanotomography to reconstruct three dimensional gland models. We integrate these morphological data with spatially resolved transcriptomic and proteomic approaches to identify the cellular and molecular machinery underlying fiber synthesis, and we couple metabolomic analyses with tensile testing to quantify the composition and mechanics of the silk. Our interdisciplinary 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.

Unveiling continent-wide migration in Odonata through unconventional approaches

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Insect migration has received considerably less attention from zoologists compared to that of vertebrates, despite its major ecological relevance in transferring large amounts of biomass between ecosystems. This gap is primarily due to methodological limitations: the small size of insects does not allow the use of technologies and approaches that have proven fundamental in understanding vertebrate migration (e.g. bird banding or satellite telemetry). Among Odonata, a significant number of species exhibit migratory behaviour—an adaptation that has likely evolved in response to seasonal changes in their aquatic breeding habitats. The biology of this order is well studied and, among insects, they are among the most conspicuous and well-known, making them ideal targets for studies on migration. However, the impossibility of directly tracking their large-scale movements necessitates the adoption of alternative unconventional approaches. *Anax ephippiger* is a tropical species that occurs seasonally in Europe and is known for its long-distance, and sometimes invasive, migrations. Despite this, very little is known about the characteristics of its migratory behaviour. This is due in part to the fact that, although it is one of the largest species in the European dragonfly fauna, tracking its large-scale movements remains challenging. In this study, we describe the spatial and temporal patterns of *Anax ephippiger* migration at the European scale using occurrence data derived from citizen science ($> 27,000$ records). The results show that the species undertakes long-distance movements ($> 5,000$ km) that follow consistent routes year after year. The migrants reaching Europe in early spring originate from Africa; these individuals reproduce in Europe, and their offspring give rise to a second migratory wave in late summer. These findings improve our understanding of dragonfly migration and highlight the need for unconventional approaches and methodologies to effectively describe their movements. Furthermore, they emphasize the conservation challenges posed by migratory insects, which exploit different areas and habitats at different stages of their life cycle and therefore require targeted conservation actions at different times of the year.

Biomineralogy: a study at the interface between petrography and marine biology

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Biomineralogy is a multidisciplinary approach investigating the complex of interactions between biological systems and lithological substrate features in marine and freshwater environments. The biotic components can recognize, select, respond to, and even utilize environmental minerals across various spatial, temporal, and biological scales.

This capacity is particularly relevant to understanding how benthic organisms colonize different substrates. To assess the potential influence of substrate type on benthic community settlement and development, we examined assemblages within the Tavolara—Punta Coda Cavallo MPA (NE Sardinia), where limestone and granite outcrops coexist within a confined area. Community diversity and structure were analyzed through photographic surveys conducted on the upper and lower surfaces of large boulders at approximately 30 meters depth.

On the illuminated upper surfaces, multivariate analyses revealed significant differences between limestone and granite sites. Limestone generally hosted a thicker and more heterogeneous coralligenous basal layer. Among the structuring species, the gorgonian *Eunicella cavolini* was more frequently found on limestone, while the sponges *Sarcotragus foetidus* and *Axinella spp.* were more abundant on granite.

Coralline algae growth and the presence of boring fauna contributed to highly three-dimensional limestone microhabitats characterized by cavities of varying sizes. In contrast, granite - virtually impenetrable - offers a more homogeneous substrate, with shaded areas and shelters formed through fracturing or rock accumulation.

Opposite patterns were observed on the shaded lower surfaces of the boulders: limestone was mostly bare, whereas granite was completely covered, likely due to differences in dissolution rates, stemming from the distinct mineral composition and texture of the two rock types. Limestone is more susceptible to dissolution, resulting in a less stable surface, thereby reducing its suitability for zoobenthic colonization compared to the more stable granite

These findings indicate that interactions between benthic organisms and substrate lithology occur on multiple levels. The lower physical and chemical stability of limestone is offset by the presence of coralline algae, which limits erosion and promotes the establishment of diverse communities. These differences may extend to higher trophic levels, influencing the distribution of mobile organisms and shaping their interactions.

Microarthropods as soil quality indicators among and beyond the timberline in Northern Apennines

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Soil biodiversity is pivotal for ecosystem homeostasis, especially in delicate high-altitude landscape. Microarthropods are effective bioindicators of soil quality due to their sensitivity to environmental fluctuations. We aim to investigate soil microarthropod quality along the upper forest–grassland ecotone in “Parco del Frignano” (Emilia-Romagna, Italy). This study is enclosed in the satellite project “BioBeRoots”, coordinated by “Ente Parchi Emilia Centrale” under NBFC.

Soil samples were collected from the surrounding of lake: Santo Modenese, Baccio, Pratignano, and Ninfa characterized by different soil erosion statuses and vegetation types (e.g. beech and conifer forests, pastures and vaccinium heathlands) all above 1300 m a.s.l. Across 2024-25, 40 stations were sampled thrice, once for the three snow-free seasons, using a standardized protocol (three soil-cores of 1L in $1\mathrm{m}^2$). GPS, altitude, vegetation type, soil temperature and relative humidity were recorded. Microarthropods were extracted using Tullgren-Berlese funnels, then preserved in alcohol for analyses, museum reference, education, and further studies. Soil biological quality was assessed through QBS-ar, Hill numbers, evenness indices, and statistical analyses.

Sampling campaigns are complete, while final sorting is ongoing. Preliminary analyses of microarthropods inhabiting soils under different vegetation types indicate that: pastures host high biodiversity and relatively high evenness but exhibit lower QBS-ar values; beech forests show moderate diversity and high evenness yet are also associated with significantly lower QBS-ar; coniferous forests consistently exhibit the highest QBS-ar values, despite hosting a slightly less even community; vaccinium heathlands tend to show variable conditions, with lower diversity and QBS-ar.

The QBS-ar discriminates significantly among vegetation types, but it should be interpreted alongside diversity and evenness metrics to capture the full complexity of pedobiological composition. Coniferous forests, though anthropogenic, prove to be valuable reservoirs of soil biodiversity. While vaccinium heathlands and beech forests display lower functional quality. Targeted management of forests and heathlands is therefore crucial to preserve biodiversity and safeguard soil-based ecosystem services with economic value.

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From shell shape to gonadal cycle: the case of *Ruditapes decussatus* population from capo Peloro lagoon (Messina, Italy)

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The grooved carpet shell clam (*Ruditapes decussatus* Linnaeus, 1758) has long been integral to the aquaculture traditions of Messina (Sicily, Italy), particularly within the Capo Peloro Lagoon, where its centuries-old breeding is part of the local shellfish farming heritage. However, the introduction of the allochthonous congener *Ruditapes philippinarum* (Adams & Reeve, 1850) into Italian waters has caused a marked decline in native *R. decussatus* populations. This shift was primarily due to the superior resilience and reproductive fitness of *R. philippinarum*, compounded by frequent hybridization events. Efforts to conserve *R. decussatus* are further complicated by the technical challenges associated with its controlled reproduction. In response to these issues, the aim of this study is to characterize a native population of grooved carpet shell clam within the Capo Peloro Lagoon. For this purpose, a total of 300 specimens were collected between September 2022 and September 2023 using a minimally invasive harvesting method. The species was first identified on a morphological basis and later confirmed by molecular analysis performed on some specimens collected. Several measurements were made in the laboratory and used to calculate the shell morphometric indices and ratios. Three size classes were determined, based on shell length (10 mm Class 1 22.9 mm; 23 mm Class 2 31.9 mm; 32 mm Class 3 48 mm). The innovative use of specific packages of R software allowed to assess the shape variation by analysing the outlines of binarized shell pictures to obtain a comparison between size classes. The Electronic Length Frequency Analysis (ELEFAN) was performed on shell measurements to estimate growth parameters, while basic histological techniques were used to assess the gonadal development. The results of shell shape analysis revealed significant differences (p ¡ 0.05) between all the size classes. Slight differences were also detected between left and right valves, suggesting possible adaptive asymmetry. Histological results and growth parameters indicate that individuals of this population regularly complete their gametogenic cycle, supporting stable recruitment dynamics. These findings not only contribute to the understanding of population structure and reproductive biology of *R. decussatus* but also provide a methodological baseline for future studies focused on the conservation and management of native bivalve species in the Mediterranean basin.

Does wing buzzing by key pollinators induce responses in *Antirrhinum litigiosum*?

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Plants and pollinators have coevolved for millions of years. This close relationship has influenced the anatomy and physiology of pollinators, enhancing their efficiency in collecting pollen and nectar. In turn, plants have evolved specific flower traits (e.g., shape, colour, scent, nectar) to attract efficient pollinators and increase their reproductive success. Our research aims to test the hypothesis that plants can sense and respond to the sounds emitted by their pollinators during flight. In this way, plants might activate the energy-demanding attraction processes only when their efficient pollinators are nearby, optimising resource allocation and increasing their fitness.
To investigate this, we first characterised the pollination network of the model plant species *Antirrhinum litigiosum* through observational surveys of four wild populations within their native range in the Iberian Peninsula, covering their entire flowering season. Then, we performed pollinator exclusion experiments to identify the key pollinators contributing to the plant's reproductive success. We correlated the identity of the visiting pollinator, as well as the number and duration of the visits to each flower, with the number of seeds produced by the corresponding fruits (i.e., seed set). Finally, we conducted a playback experiment on lab-grown plants to assess changes in their nectar composition after different vibroacoustic treatments.

A. litigiosum was visited by more than 40 insect species from four different orders, which interacted with the flowers in various ways (e.g., pollinators, nectar robbers, florivorous). Single visits by different pollinators resulted in varying amounts of seeds produced by the fruits, and a higher number of visits to a flower consistently increased seed set. We used the data on pollinator effectiveness to select the vibroacoustic stimuli for playback experiments. Preliminary results showed that plants exposed to the buzzing sounds of a key pollinator produced more nectar with a higher sugar content than those exposed to control sounds (background or pink noise).

These findings support the idea that attracting efficient pollinators and increasing their visitation rates can significantly benefit the plant's reproductive success. To achieve this, plants may have evolved the ability to sense and respond to the vibracoustic stimuli emitted by their pollinators during flight, activating the attraction mechanisms at the optimal time.

Unraveling the role of microbial communities in host response to chemical stress

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The microbiota of marine invertebrates is progressively recognized as a key player in modulating host responses to environmental stressors, including chemical pollution. The microbiota of Manila clams (*Ruditapes philippinarum*) inhabiting the highly contaminated area of Porto Marghera (Venice Lagoon) show consistent enrichment in detoxifying bacterial taxa and functional pathways linked to xenobiotic degradation. This suggests a possible adaptive interaction between host and microbiota in polluted environments. To explore the contribution of resident microbiota in modulating clam responses to chemical exposure, an innovative and previously untested microbiota transplantation approach was developed. Clams from a commercial hatchery were subjected to microbiota depletion via antibiotic treatment, then recolonized using two distinct methods: (i) cohabitation with adult donor clams from Porto Marghera and (ii) exposure to a homogenate prepared from donor soft tissues. Following transplantation, clams were exposed for one week under laboratory conditions to a mixture of metals at environmentally relevant concentrations (Cd 0.5 µg/L and Hg 0.03 µg/L), reflecting those frequently detected in Venice Lagoon. Digestive gland tissues were collected for host transcriptomic analysis (RNA-seq), and microbial communities were characterized using 16S rRNA sequencing. This intricate experimental design allows the assessment of both the efficacy of microbiota transfer and its influence on host gene expression in response to metal exposure. By integrating microbial and host-level analyses, this study aims to decode the functional role of transplanted microbiota in the modulation of clam tolerance to chemical stress, ultimately contributing to the understanding of microbiota-mediated adaptation mechanisms in marine invertebrates exposed to anthropogenic stressors.

Targeted seminal toxin delivery for mosquito suppression

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The global expansion of *Aedes albopictus*, fueled by climate change, urbanization, and global trade, presents a growing public health threat due to its competence in transmitting arboviruses such as dengue and chikungunya. Conventional control methods have shown limited long-term effectiveness, underscoring the need for sustainable and targeted solutions. In this context, and similarly to the the Toxic Male Technique (TMT) strategy, the TOXMOS project (TOXic Males for MOSquito Control) aims to develop a novel biocontrol strategy in which genetically modified males deliver seminal fluid-borne toxins to females. Once transferred, toxins disrupt the female reproductive capacity and reduce their lifespan, eventually suppressing vector populations and pathogen transmission. Notably, mathematical models highlighted this strategy has superior efficacy over classical Sterile Insect Techniques (SIT), particularly for polyandrous species like *Ae. albopictus*.

To achieve male accessory gland (MAG)-specific expression, two PiggyBac-based constructs were generated using the *Aedes aegypti* AAEL010824 promoter: one encoding eGFP alone, and the other including a signal peptide (SP) sequence to facilitate protein secretion. Transgenic lines were established through embryo microinjection and maintained under standard insectary conditions. Expression analysis by confocal microscopy showed successful eGFP localization into the MAGs, with the SP-enhanced construct leading to fluorescence accumulation in the glandular lumen. However, RT-PCR revealed off-target expression in other tissues, suggesting promoter leakiness. To address this, a binary expression system based on the *Neurospora crassa* Q-system is under development. This modular platform includes driver lines expressing the QF2W transcriptional activator under MAG-specific control, and effector lines in which eGFP—or future toxins—is regulated by a QUAS enhancer. In hybrid males, the effector gene will be activated specifically in the MAGs. Ongoing efforts also include screening additional promoters and testing genomic insulators to mitigate positional effects.

Together, these findings establish a solid foundation for a highly specific, genetically engineered biocontrol tool in *Ae. albopictus*, with broad implications for vector management and the prevention of mosquito-borne disease outbreaks.

The migration of a critically endangered damselfly calls for habitat-specific conservation actions

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Among Palaearctic Odonates, adult overwintering is an evolutionary feature exclusive to the genus *Sympecma*. In Italy, *Sympecma paedisca* preferentially breeds in the rice field district of Piedmont, while heathland fragments between Piedmont and Lombardy are its main overwintering habitat. It is not clear how this Critically Endangered species move between these two disjunct areas, located kilometres apart. To shed light on this, we investigated: 1) how habitat composition affects the abundance of the species at a landscape scale; and 2) its fine-scale habitat selection, accounting in both cases for the season. To this aim, we designed 60 transects located in the range of the species along a gradient encompassing heathland fragments and the surrounding agricultural matrix. We counted the species within 2 m from each transect and assessed habitat composition within 50 m. Then, each *Sympecma* location was attributed to a habitat (within 2 m) and then compared with random locations according to a use-availability design. Transects were repeated five times between August 2023 and April 2024 to cover the entire non-breeding phase.

On a 50-m scale, the abundance of the species increased significantly as the cover of well-conserved heathland increased, and this relationship does not vary between seasons. In contrast, the effect of farmland cover varied significantly between seasons, being positive in summer, strongly negative in autumn and slightly negative in winter and spring. The habitat selection analysis showed that in summer, the species used grassy field and road margins in cultivated areas, abandoning them as the winter season progressed. On the opposite, an increasing positive selection trend for heathlands was observed, while degraded heathlands were avoided. These results indirectly support the hypothesis that, in late summer, the species migrate from its breeding grounds to the overwintering heathlands, following the network of grassy margins found in the agricultural matrix. Individuals that survive winter, come back in spring following the same grassy margin network. Conservation efforts should be tuned on the different needs of the species throughout its life cycle: well-preserved heathlands are crucial for the overwintering of the species and thus require active management to limit forest encroachment. In parallel, grassy margins in agricultural landscapes must be preserved to maintain connectivity between wintering and breeding grounds.

Morfo-functional analysis of *Paracentrotus lividus* stereom for bioinspired material design: an interdisciplinary approach

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A crucial role in the evolutionary success of Echinoids has been played by their resistant and versatile endoskeleton, composed of a porous 3D calcitic network called stereom, which combines strength and versatility. Its well-defined structure-function relationship offers valuable bioinspiration for biomimetic applications in engineering, architecture, and materials design.

Our project adopted an interdisciplinary and innovative approach, integrating expertise from zoologists and mathematicians (Università degli Studi di Milano) paired with mechanical engineers (Politecnico di Milano). Constant communication among these disciplines enabled mutual guidance, aligning biology-driven morphological analyses, mathematical modelling, and design development. The research aimed to deepen morpho-functional knowledge of the stereom in *Paracentrotus lividus* (Lamarck, 1816), comparing three interambulacral plate types (aboral, ambital, oral) across different size classes (ages), ranging from 1 to 6 cm in diameter.

Individual plates were isolated and longitudinally abraded to expose the median section of the primary tubercle. A total of seven stereom types were identified and analyzed in detail: their porosity and trabecular organization were investigated through 2D imaging using Scanning Electron Microscopy (SEM) and 3D modeling based on scans performed at the Synchrotron. Using ImageJ software, porosity, trabecular orientation, and stereom thickness were quantified. Compression tests were carried out to examine the mechanical properties of the stereom, particularly focusing on the tubercles, which distribute point load evenly across the plate, providing a strategic combination of lightness and strength. The results show a statistically significant difference in porosity percentages among the investigated stereom types across various sea urchin diameter classes. However, these differences are not statistically significant among the different plate types. Mechanical tests reveal that among the three types of plates investigated, the primary tubercle of the oral plate is the most resistant. Moreover, additional data confirm that the tubercle stereom behaves similarly to a lattice structure, combining lightness with high impact resistance. Therefore, this study will provide valuable insights into the morpho-functional and mechanical properties of *Paracentrotus lividus* stereom, laying the basis for developing bioinspired structural materials.

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Photoreceptor control of animal growth and lifespan via evolutionary conserved molecular pathways

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Natural light is severely affected by human impact on Earth, yet little is known about the roles light receptors have outside vision and rhythmic processes, despite their tremendously wide abundance. Here we show that loss-of-function of the *light-receptive cryptochrome* (*l-cry*) in marine bristleworms significantly increases lifespan and adult size, similarly to wild-types reared in constant darkness. Quantitative transcriptomics revealed hormonal players crucial for invertebrate and vertebrate sexual development and reproduction affected in *l-cry* mutants. These include *nr0b1/2*, ortholog of *dax-1* (*nr0b1*) and *shp* (*nr0b2*), long considered vertebrate novelties. Depending on moon-phase, *nr0b1/2* is up- or down-regulated in *l-cry* mutants. Matching the complex regulation, loss of *nr0b1/2* function partially recapitulates *l-cry* phenotypes. Molecularly, *Platynereis* Nr0b1/2 affects steroidogenic and other endocrine pathways, nuclear receptor signaling, and transcription factor orthologs, involved in sexual developmental, reproductive, and timing processes in other organisms. Thus, our study suggests profound and quite direct effects of light on adult animal life-time, likely at least in part by conserved endocrine pathways involved in sexual maturation and reproduction in annelids and vertebrates.

Passive acoustic monitoring of pollinators: integrating IoT sensors and deep learning for species identification

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Pollinators, such as bees, hoverflies, and butterflies, serve as key bioindicators of biodiversity, providing insight into overall ecosystem health. Many species especially wild bees and hoverflies, are Data Deficient according to the IUCN, emphasizing the need for improved monitoring and conservation efforts. Emerging technologies can help close this information gap by providing enhanced transparency, traceability, and measurable assessments. This study focuses on the development of passive acoustic monitoring (PAM) techniques for the Apoidea and Syrphidae families through the integration of IoT-based bioacoustic sensors. These devices aim to capture species-specific flight sounds supporting automated identification via machine learning models, thereby reducing the dependency on physical specimen collection and associated mortality. While acoustic recognition has shown promising results for some wild bee taxa its broader application remains limited, particularly for hoverflies, which lack large acoustic datasets. To address this challenge, we created a reference acoustic library composed of 549 recordings representing 53 different species, with the purpose of training and validating the identification algorithm. Simultaneously, we installed IoT-enabled recording devices at six monitoring locations within the metropolitan area of Turin (TO), capturing flight-generated acoustic signals while conducting conventional surveys (e.g., pan traps and transects) to allow comparative analysis. Although these sensors do not perform onboard species identification, the collected sound data are continuously uploaded to the cloud via 4G for further processing. Buzz frequency data were subsequently compared with abundance data derived from conventional sampling methods, revealing a consistent trend between the sensor outputs and pan trap results.

The identification model, built using a ResNet50 Convolutional Neural Network (CNN) architecture in MATLAB, has shown high classification accuracy among species. Moreover, by segmenting flight-related acoustic emissions into distinct phases such as hovering, approaching, landing, and departure, the model's predictive performance has been further enhanced. Once the algorithm is fully implemented into the field sensors the system will enable extensive-scale PAM, allowing the acquisition of biodiversity data that is both spatially distributed and temporally continuous, representing a significant advancement in ecological monitoring.

Sessione
Nuove frontiere della Zoologia: innovazione e sinergie interdisciplinari

Poster

Exploring the biodiversity of tube-dwelling coral simbionts from the Faafu atol (Maldives)

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Coral reefs are highly productive ecosystems. Species associated with coral reefs are estimated in over than a million, of which about 90% are still not described. The Maldives islands are characterized by a very diverse marine faunal assemblage associated with one of the world's most biodiverse coral reef ecosystems. This study aims to investigate the diversity of tube-dwelling coral symbiotic fauna associated with reefs around the Faafu atoll (Maldives archipelago), focusing on their host prevalence and dominance. Two underwater visual census campaigns were performed in different sites near Magoodhoo island, Faafu Atoll (Sep 2024, Feb 2025), meanwhile sampling the live and dead hard coral portion hosting the symbiotic organisms. Photo-video recording of domicile openings of the different animal taxa was carried out, to avoid over-sampling and to set a non-impacting method to identify the coral reef's associated animals. Samples were transported to MaRHE Center laboratory in Magoodhoo for a first morphological identification of associated macrozoobenthic fauna and photo reporting of their associated bores. Samples of corals and associated animals were stored in ethanol and transported to the Messina University laboratories for molecular identification. Porites and Echinopora represented the most monitored coral genera, while Plesiastera and Pleuractis genera were found less inhabited and therefore sampled. Interestingly, even dead coral resulted in housing epibionts, especially for the relationship between crustaceans and dead Echinopora coral. The preliminary analyses on 44 collected coral samples showed the presence of about 110 specimens of several taxa belonging to three main phyla, Crustacea (61%), Anellida (18%), and Mollusca (21%). The most represented orders resulted in Decapoda, Sabellida, and Mytilida. The crossing of both sampled and underwater photo-videographic material analyses helped to create a taxa-domicile opening relation, indirectly identifying 181 additional specimens in total (53% Crustacea, 27% Anellida, 20% Mollusca). Photographic determination is currently ineffective for species discrimination, but can certainly be improved. Further studies are needed to assess symbiotic species identification, type of interaction, and species-specific relationships with corals. Focusing on these relationships in changing environments is essential and these data shed light on interesting insights, achievable through non-invasive sampling methods.

Flyer or percher, bigger or smaller - a study on factors producing phenotypic diversity in dragonfly wings

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Flight is a key innovation in insects, driving immense morphological and functional variation in wings. Yet how this diversity arises under the combined influences of biomechanics, ecology, genetics, and phylogeny remains unclear. Dragonflies (Anisoptera), among Earth's oldest and most agile fliers, epitomize this puzzle: their wings vary widely across species due to both ecological factors and allometric scaling.

We investigate this topic in an integrative fashion combining molecular phylogenetics, geometric morphometrics, and phylogenetic comparative methods. Our goal is to determine not only whether different sources of variation shape wing morphology, but also their relative contributions, the similarity of the phenotypes they produce, and how they influence macroevolutionary patterns. We focus on two primary flight strategies – "perchers" (sit-and-wait predators) and "flyers" (sustained-flight predators) – and how these relate to shape variation and allometric trends. Using a dataset of over 2,000 dragonfly wings, we characterised morphological variation with a set of 42 landmarks and semilandmarks. Then, using phylogenetic comparative approaches we tested hypotheses about allometric and non-allometric contributions to wing shape and evolutionary dynamics. With unprecedented resolution in terms of number of species, we reveal shape differences consistent with flight strategies: flyers tend to have larger, more elongated wings, presumably to maximize efficiency, whereas perchers display shorter, robust wings for rapid bursts and hovering. Crucially, these differences partially aligned with allometric variation. In conclusion, our findings highlight the interplay of intrinsic (genetic correlations, previous evolutionary history) and extrinsic (adaptation to different food sources and hunting strategies) factors in shaping dragonfly wing morphology. Our study also demonstrates the power of advanced techniques and models to understand macroevolutionary patterns and lays a solid foundation for future investigations of form, function and evolution across Anisoptera.

Integrated surveillance of arboviruses in Liguria: The regional plan for vector and pathogen monitoring

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Since 2024, the Liguria Region has implemented a regional plan for arbovirus surveillance, entitled "Piano regionale di prevenzione, sorveglianza e risposta alle arbovirosi" which includes coordinated actions targeting both vector monitoring and pathogen detection in humans and other animals acting as hosts or reservoirs.

The plan provides for a widespread monitoring network during the vector activity risk period, from May 1 to November 30 each year. Twenty-five fixed monitoring stations are in place, with additional stations activated in response to potential epidemiological emergencies. Sampling of culicid dipterans is performed using BG-Sentinel traps with BG-Lure and carbon dioxide attractants; water samples from drains and catch basins are also occasionally analyzed. Species identification is conducted at the laboratories of the Experimental Zooprophylactic Institute (IZS) of Piedmont, Liguria, and Aosta Valley, where molecular techniques are used for pathogen detection.

All four provinces in Liguria are considered high-risk areas, partly due to their proximity to France, where several Dengue cases have been reported in recent years. The collaboration among physicians, zoologists, and veterinarians has led to the detection in Liguria of invasive species such as *Aedes koreicus* and *Aedes japonicus*, in addition to the well-known *Aedes albopictus*, and to the timely implementation of containment measures for imported Dengue cases in returning travelers.

Constant vector surveillance and the ability to implement immediate containment actions are essential tools to prevent the emergence of autochthonous outbreaks, as already observed in other Italian regions.

The ontogeny of vocal rhythms in a non-human primate

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Some primates communicate by using changes in sound frequency and rhythm to convey information. However, are these rhythmic patterns innate, or do they develop over time? Species producing series of calls showing rhythmic categories corresponding to small-integer ratios, where sounds and pauses follow simple numerical relationships, are rarely observed in non-human mammals. In this study, we examine rhythm in the songs of the lemur *Indri indri*, focusing on how rhythms corresponding to small-integer ratios emerge throughout development. From 2010 to 2022, we recorded the songs of 62 individuals (29 females) from different age classes (0–2 years, 3–4 years, and adults) in the Maromizaha New Protected Area, Madagascar. We analyzed individual contributions to group songs, measured the intervals between the onset of each note (inter-onset intervals), and calculated rhythmic ratios between adjacent intervals. Our findings show that isochrony - a regular 1:1 ratio, like a ticking clock - is a consistent feature of indri songs across all ages and sexes. Furthermore, two additional small-integer ratios (1:2 and 2:1) appear during development, with distinct patterns in males and females. These findings suggest that indris undergo rhythmic development, likely reflecting an increasing ability to combine and structure vocal elements in more complex ways.

Functional anatomy of the eversible pharynx of *Glycera tridactyla* (Annelida: Glyceridae): A micro-CT contribution

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Polychaete annelids are characterised by a wide array of pharyngeal organisations, associated with different feeding strategies, which might represent useful inspirations for next-generation soft robots. Members of the family Glyceridae have a particularly interesting eversible pharynx, occupying up to one third of their body length and armed with mineralised jaws connected to venom glands, making it a particularly interesting model for the inclusion of hard elements in soft structures. Within the framework of the EIC Pathfinder Open project MAPWORMS (grant agreement No 101046846)In this study, the functional anatomy of *Glycera tridactyla* Schmarda, 1861 was reconstructed through micro-computed tomography (micro-CT) using several individuals fixed in different positions. Results from micro-CT scans were compared with historical reconstructions based on dissection, giving substantially consistent results, and confirming the usefulness of this technique for the reconstruction of the internal anatomy of small invertebrates.

Morpho-functional analysis of the madreporite plate in *Paracentrotus lividus*: implications for biomimetic design

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The sea urchin *Paracentrotus lividus* represents a valuable model for biomimetic investigations due to its distinctive morpho-functional adaptations. Among these, the madreporite plate is a key element of the water vascular system, located within the apical system and pierced by a network of canals that facilitate the intake of seawater into the organism. The aim of this work was to study the morpho-functional organization of the madreporite to obtain innovative constructional concepts and ideas for the development of design objects.

Madreporite plates were dissected from *P. lividus* specimens of different sizes (1-6 cm). Samples were processed and examined using different morphological techniques: scanning electron microscopy (SEM) to investigate both skeletal and cellular components; histological analyses to examine the detailed cellular and ultrastructural organization of the canals; micro-CT analyses coupled to mathematical modelling to evaluate the overall the 3D inner organization of the canal system. The obtained results indicated that, externally, madreporite pores display a constant diameter, regardless the individual's size, but their number increases proportionally with animal diameter, thereby expanding the effective filtering surface. Internally, the madreporite is organized into two distinct regions. In the upper region, canals follow a predominantly parallel course, they are lined by a cylindrical epithelium provided with a dense network of cilia and the actual lumen is small. In the lower region canals curve and converge toward a common collecting area, the lining epithelium is almost cubic, cilia are apparently less numerous and the lumen is larger than in the upper region, thereby facilitating seawater flow. Additionally, anastomoses between adjacent canals can be also observed. Overall, these data suggest that the madreporite functions as an extremely effective filtration system (against bacteria or nano-microparticles) and rely on a complex fluid transport mechanism which contribute to the regulation of pressure and volume within the water vascular system. By means of a biomimetic approach, these constructional principles served as a basis for the design of bioinspired functional objects, such as fruit bowls or other fluid-regulating containers, therefore allowing a conceptual transfer between extremely distant disciplines as Zoology and Design.

New insight into the crayfish *Procambarus clarkii* (Girerd, 1852) (Crustacea, Cambaridae): molecular and morphometrical tools to describe the case of sardinan population

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Invasive Alien Species (IAS) are ranked as the second greatest cause of species endangerment and extinction. Among them, *Procambarus clarkii* (Girard, 1852), originally from America and voluntarily introduced in the Mediterranean area for aquaculture and pet trade scopes, is an extremely invasive crayfish which is leading European freshwater ecosystems to a global biodiversity crisis. One of the causes of biodiversity loss linked to the crayfish invasion is the disease called "crayfish plague," spawned by the parasitic fungus *Aphanomyces astaci*, naturally carried by *P. clarkii.*

The invasive North American crayfish species appears, in fact, to tolerate and resist this infection as result of a balanced relationship arising from the coevolution with *A. astaci.* As a result, autochthonous crayfish populations are severally hit by the infection, while the alien one can overcome it. For this reason, early detection of this harmful crustacean, together with the study of its biological life cycle, is crucial to develop effective management plans aimed at minimizing its impact on the local fauna. Thanks to molecular taxonomy and pathogens molecular diagnostic techniques, a southern Sardinian population of *Procambarus clarkii* was studied. Molecular analyses allowed the taxonomic attribution of individuals and the diagnosis of the presence of *A. astaci* in their tissues (if any). Furthermore, with the use of morphometry, evidence of the presence of two biological alternative morphotypes in *P. clarkii* specimens was found. The relative growth of the body part of interest (Chela length, ChL), with respect to a reference dimension (Carapace length, CL), was examined through the allometric growth equation. Using the same method, applied to the secondary sexual character (Gonopod length), the Size at Onset of Maturity, from a morphometric point of view, was provided for the first time.

RESULTS: All the individuals were molecularly identified as *P. clarkii* and none tested positive for *A. astaci.* The presence of the two alternative morphotypes, linked to the reproductive period, was statistically proven for the first time for Sardinian red swamp crayfish. The Size at Onset of Maturity (SOM) was estimated between 35 and 37.1 mm CL.

Soil biodiversity under AgroPhotoVoltaic Systems: insights from arthropod communities

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The increasing implementation of AgroPhotoVoltaic (APV) systems, which combine solar energy production with agricultural practices, offers a promising solution to land-use conflicts between food and energy needs. While the effects of APV on crops and broader environmental parameters have been explored, impacts on soil biodiversity—especially soil arthropods—remain under-investigated. These organisms are essential for nutrient cycling, soil structure formation, and pest control, and respond sensitively to microclimatic and soil property changes induced by panel shading.
This study, conducted within the SUNRISE PNRR project at an APV site in Borgo Virgilio (northern Italy), assessed soil biodiversity under two types of solar trackers with different shading levels: standard (15%) and expanded (40%). Four treatments were compared: crops shaded by standard or expanded trackers, crops in full sun, and a grass-covered control area. Wheat and tomato were the selected crops; wheat was studied under all treatments, tomato under three (excluding expanded shading). Soil samples were collected over multiple growing seasons and analyzed for soil pH, organic matter (SOM), respiration (CO), and arthropod communities.
Results revealed that soil biological quality, CO emissions, and arthropod diversity were more influenced by crop type, and seasonal dynamics than shading intensity. Control plots generally supported greater QBS-ar values and higher Collembola richness and evenness, especially in wheat. Positive correlations were found between QBS-ar and SOM, CO emissions, and soil temperature, while pH showed negative correlations. Arthropod community composition shifted with crop phenology, with indicator species such as Thysanoptera and Polyxenida in early wheat, and Isopoda in late tomato stages. FAMD analyses highlighted that treatment-driven variations in SOM, moisture, and temperature contributed to distinct community structures, particularly among Isotomidae and Entomobryidae.
These findings emphasize the strong interplay between crop management, soil properties, and soil fauna under APV systems, highlighting their potential ecological impact. Understanding these dynamics is key to developing sustainable agroecosystems that balance productivity with zoology and biodiversity conservation.

Integrating zoological knowledge into river management: the role of fish ecology in the IDRAIM model

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Fish species play a key role in river and transitional ecosystems, and their biology is closely linked to sediment and vegetation dynamics. Understanding these interactions is essential to guide sustainable engineering choices. The European eel (*Anguilla anguilla*), a declining euryhaline species, is an emblematic case of complex migration (homing) and high sensitivity to habitat modifications.

In this study, zoological knowledge is integrated into an interdisciplinary river management project that applies the IDRAIM model, developed by ISPRA for hydromorphological assessment and solid transport analysis. By analyzing fish and plant communities as well as collecting environmental data, we provide essential inputs to assess river ecological status and support low-impact interventions.

In the case of Rio Geremeas, a small stream in south-eastern Sardinia, we applied non-invasive techniques (electrofishing, floating traps for glass eels, nets) for fish communities, while for riparian vegetation sampling, we used a physionomic-structural approach easily applicable for management purposes, with information on the presence and impact of "transformer" invasive alien species. To collect environmental data, we used multiparameter probes. This Mediterranean site, highly influenced by seasonality and environmental fragmentation, showed a reduced presence of fish fauna, likely due to anthropogenic pressure and climate variability. Based on our findings, we proposed a nature-based management of the river mouth opening to support natural processes since any classical intervention, like storm drain in the floodplain, would further disrupt ecological connectivity and water availability.

In addition, we propose the containment or, where it is possible, eradication with conservative methods for sediments (e.g. cover the plots with black geotextile tarp) of invasive alien plant species *Arundo donax*, *Eucalyptus camaldulensis* and *Acacia saligna* that reduce animal and plant diversity by compromising the functionality of the riverine ecosystem.

This case study highlights how integrating biological data is crucial to enhance the IDRAIM model's effectiveness in evaluating sediment transport, while simultaneously improving environmental quality and reducing hydrogeological risk. It demonstrates the value of an ecosystem-based, interdisciplinary approach in modern river management.

MonOrniTech: innovative technologies for ornithological monitoring in the Laghi Lungo and Ripasottile Reserve

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Italian wetlands play a crucial role in bird conservation, serving as breeding, resting, and feeding habitats for numerous species, including many threatened by anthropogenic and environmental factors. In this context, MonOrniTech was born: a pilot project aimed at developing and testing innovative technological solutions for participatory bird monitoring in protected areas, with particular focus on the Laghi Lungo and Ripasottile Nature Reserve, a wetland of community importance in the province of Rieti. The objective is twofold: on one hand, to improve the quality and continuity of ornithological monitoring through digital tools; on the other, to encourage the active involvement of citizens in citizen science activities.

The project foresees the integrated use of various non-invasive methodologies: internal and external environmental sensors, camera traps, digital applications for field data collection, supported by training activities for volunteers and birdwatching enthusiasts. The participatory component is central, with field days dedicated to guided observation, skill transfer, and sharing of collected data. An exploratory phase is currently underway, including the selection of installation sites, fine-tuning of devices, and the definition of standardized protocols. The MonOrniTech project is funded by the European Union - Next Generation EU (Mission 4 Component 1 CUP I13B24000010002).

Although quantitative results are not yet available, early qualitative feedback shows strong interest from the local community and a good response to outreach initiatives. Public events such as "Spring in the Reserve: discovering the MonOrniTech project," held in April 2025, allowed testing of field activities and the collection of preliminary observations on target species and participation levels.

In conclusion, MonOrniTech aims to serve as a replicable model for participatory bird monitoring, based on the integration of technology and citizen engagement. The next phases will include the analysis of collected data, evaluation of the effectiveness of the employed technologies, and dissemination of results to managing bodies and the scientific community.

Workshop
Etica, Benessere animale e 3R

Comunicazioni

The legal protection of animals in scientific experimentation

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Animal experimentation represents one of the most delicate and controversial areas in balancing scientific progress, biomedical research needs, and the protection of non-human living beings. This presentation offers an in-depth analysis of the current legal framework governing the use of animals for scientific purposes, highlighting the main European and national regulations as well as the underlying ethical and bioethical principles. At the European level, Directive 2010/63/EU is the key normative reference and promotes the principle of the so-called "3Rs": Replacement (substitution of animals with alternative methods), Reduction (reduction in the number of animals used), and Refinement (improvement of techniques to minimize suffering), guiding research toward more sustainable practices that respect animal welfare.

In Italy, Legislative Decree no. 26/2014 transposed the directive with some stricter provisions, introducing additional restrictions such as the ban on breeding dogs, cats, and non-human primates, as well as limitations on their use. These measures have sparked intense debate among the scientific community, institutions, and animal rights organizations. The presentation also delves into the legal concept of animals throughout history and the progressive recognition of animal welfare as a legally relevant value, particularly in light of the recent amendment to Article 9 of the Italian Constitution, which extends environmental protection to include animals explicitly as entities worthy of legal protection.

The role of the bodies responsible for overseeing and authorizing experimental protocols—such as the competent authorities—is examined, along with the responsibilities of those involved, including researchers, veterinarians, and institutions, with regard to transparency, training, and reporting obligations. Particular attention is given to the comparison between scientific needs, the development of alternative methods, and the growing ethical awareness of public opinion.

The overarching goal is to stimulate critical reflection on the effectiveness of the legal protection of animals in laboratories, assessing the future evolution of legislation, also in light of recent jurisprudence and the position of some European institutions toward the gradual phasing out of animal experimentation, in favor of a more ethical, sustainable, and responsible science.

Welfare and care of the laboratory animals: new milestones achieved in the facilities

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Since the enactment of the European Directive 2010/63/EU on the protection of animals used for scientific purposes, the activities carried out in the animal facilities are conducted in full compliance with the 3R principle (Russell & Burch, 1959) with the aim to reduce the number of animals involved to a minimum, compatible with the scientific goals; to refine the housing methods and experimental techniques in compliance with the biological needs of the species; to replace the animal model, where possible.

To ensure the proper regulation and welfare of research animals, the standards of laboratory animal welfare (how animals should be correctly cared for) are continuously updated as the Commission delegated directive (EU) 2024/1262 of 13 March 2024 regarding the requirements for establishments and for the care and accommodation of animals.

In Italy the legislative context has been implemented by the Decree 26/2014 and new training obligations have recently been identified by the Ministry of Health according to the Decree of 5 August 2021 and the Decree of 18 March 2022 for all personnel who work with animal models.

Such personnel must be adequately trained and must know the basic and specific biology of the single species in relation to anatomy, physiological characteristics, reproduction, genetics and genetic alteration; animal behaviour (ethology), breeding and enrichment; management methods and procedures specific to the species bred.

The competence of personnel is assessed by the Animal Welfare Body composed of the designated Veterinarian (DV), the Scientific Member (SM) and the Animal Welfare Officer (RBA). The RBA, a completely new role recently introduced, must have in-depth knowledge of Animal Biology to ensure the "well-being" of the animals and the right accommodation of the animals as well as the activity and skills of the technical and research personnel who work with the animals.

The specie-specific skills of the staff become even more important since the use of emerging animal models, i.e. species that are currently recognised as having less neurological complexity than those protected by legislation, is constantly increasing and represents a new and promising alternative in the field of animal testing (partial replacement).

Invertebrates and Humans: perspectives from Science, Ethics, and Policy

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Recent advances in cognitive ethology, neurobiology, and evolutionary biology increasingly challenge anthropocentric paradigms that have historically denied sentience to invertebrates. Accumulating evidence reveals complex cognitive and behavioral capacities across several invertebrate taxa, particularly cephalopods, hymenopteran insects, and decapod crustaceans. These organisms show adaptive problem-solving, long-term memory, behavioral flexibility, nociceptive responses consistent with pain perception, and signs of metacognition. Such findings support a paradigm shift toward viewing consciousness as a graded, biologically distributed phenomenon rooted in diverse neurophysiological substrates.

Within this framework, the ethical implications are profound. Recognizing sentience in invertebrates requires extending moral consideration beyond vertebrates. Applying the precautionary principle amid scientific uncertainty compels us to treat invertebrates as potential subjects of suffering, requiring refinement of experimental protocols and husbandry practices. This aligns with the 3Rs—Replacement, Reduction, and Refinement—promoting alternatives to invasive methods, minimizing animal use, and mitigating distress.

From a policy perspective, this evolving scientific and ethical landscape is shaping legislation unevenly. Cephalopods have been protected under Directive 2010/63/EU since 2013, while other taxa, such as decapods, have gained growing recognition in countries like the UK and Switzerland. In 2025, Italy amended its Penal Code by introducing Title IX-bis, explicitly recognizing animals as sentient beings and subjects of rights not limited to vertebrates, opening the door to protecting invertebrates where scientific evidence supports it.

Practical implications include that boiling crustaceans alive may be classified as mistreatment, insect farming may require minimum welfare standards, and cephalopods—already protected in research—could receive broader safeguards. This affirms that where there is sentience, there must be protection, regardless of taxonomy.

This interdisciplinary analysis highlights the scientific, ethical, and political challenges in reassessing invertebrate welfare. It advocates combining rigorous research, ethical deliberation, and policy reform to advance humane treatment of invertebrates, contributing to a redefinition of human-animal relationships and biodiversity conservation.

Rethinking Invertebrate Research: where do we draw the line between use and protection?

Cinzia Chiandetti[1]

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For decades, even complex vertebrate species such as birds were regarded as mere “reflex machines,” deemed incapable of flexible or sophisticated responses due to their brain being organized into nuclei rather than a layered cortex. Some of these species, fundamental to human nutrition worldwide, are unfortunately still exploited in intensive farming, with ethical, environmental, and health-related consequences.

However, as early as 2002, the international forum on avian brain nomenclature (Reiner et al., 2004) highlighted how mistaken this view was: with a different neural architecture, birds turned out to be capable of complex cognitive functions comparable to those of supposedly more evolved organisms, to the point of being described as “feathered apes” (Emery, 2004). This emblematic case prompts reflection on two crucial situations today.

The first concerns the widespread belief that complex cognition necessarily requires a centralized nervous system. Jellyfish often fall victim to this bias, commonly labeled “brainless” due to the radial and decentralized organization of their nerve net. This prejudice is reinforced by the scarcity of targeted behavioral studies. Yet, in juvenile *Aurelia* spp., we have observed behavioral markers of cognitive processes, such as short-term memory and novelty preference, challenging the notion of a complete absence of cognitive abilities (Agrillo et al., accepted).

The second concerns *Tenebrio molitor*, an insect promoted as a sustainable protein source (EFSA NDA, 2021) and a potential protagonist of future intensive farming. Currently lacking specific regulatory protections, *T. molitor* has demonstrated the ability to recognize and prefer more advantageous alternatives when motivational values of stimuli change, suggesting forms of intentional (or at least goal-directed) behavior that deserve immediate attention (Dissegna et al., 2024). This is crucial to avoid repeating, with insects, the same ethical issues - and associated impacts on animal welfare and human health - already observed in poultry farming.

These findings urge a profound reconsideration of the criteria by which we define cognitive complexity and, consequently, the boundaries between the use and protection of invertebrates. Ignoring their cognitive potential risks not only perpetuating scientific prejudices but also jeopardizing the recognition of their welfare rights, precisely at a time when new species are entering global production chains.

Wellbeing of fish used in scientific research

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The professional and technical skills required to work as figure a), b), c) and d), according to the article 23, of the Italian Legislative Decree 26/2014 must be certified as per the Italian Ministerial Decree of the 5th of August 2021, article 6 and the Italian Directorial Decree of the 18th of March 2022, article 3. The certification is obtained after the completion of specific mandatory courses. An example is the course “The use of different species of fish in scientific research” (i.e., sea bass, Salmonidae, sea bream, sturgeons and others). These courses must include the following modules and topics:

- Module 3.1: Basic and appropriate biology – species-specific
- Module 4: Animal care, health and management – species-specific
- Module 5: Recognition of pain, suffering and distress – species-specific
- Module 7: Minimally invasive procedures without anaesthesia – species-specific

All the personnel must be fully trained to accomplish their specific duties.

The Italian Ministerial Decree of the 5th of August 2021 outlines all the steps required to obtain the appropriate certifications, maintain the certifications over time and demonstrate the acquisition of the appropriate skills. Being in possess of the proper certifications is mandatory for all the personnel working as:

a) carrying out procedures on animals;
b) designing procedures and projects;
c) taking care of animals;
d) sacrificing animals.

Moreover, a Designated Doctor in Veterinary Medicine and an Appointed Officer in charge of Animal Care and Wellbeing must always be present.

When not under experimental procedures, all animals must be maintained under appropriate conditions to guarantee their wellbeing.

Swimming our way through science: the zebrafish as a research model

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The use of the zebrafish (*Danio rerio*) as a research model is gradually and continuously growing. Nowadays, the zebrafish is considered one of the most complete and flexible models in a variety of research fields: biomedical, cardiovascular, neurodegeneration, behavioural and even drug screening. This is all thanks to several specific features: embryos are transparent, which allows for better visualisation of organs and tissue development, develop all major organs within the first 5 days of life and adults have high reproduction and fertilisation rate. However, it is important to keep in mind that the zebrafish can't completely replace mammals just yet, but is considered a good alternative method that helps to drastically reduce the number of higher animals used in the scientific research. In this context, the zebrafish allows to fulfil the concept of *Reduction*, since the use of embryos helps to reduce the number of adults used, and *Replacement*, since the embryos are not regulated by the Directive 2010/63/EU and the Italian D.lgs 26/2014 due to the low neural development. It is also important to remember that the 3Rs concept also applies to the zebrafish: considering that the adults can be used for research purposes, their number should be kept to the minimum required to obtain a statistically significant result. It is also necessary to use refined techniques that can limit the amount of distress and pain caused to the animal as much as possible. Finally, even in this case, the use of embryos within 120 hours post fertilisation instead of adults is considered a replacement method. In conclusion, the zebrafish is leading the way to new possibilities and will continue to do so in the future, allowing for a gradual decrease in the number of mammals or other animals used in the scientific research.

Adaptive evolution and individual ontogenetic trajectory: the biological dimensions of welfare

Monica Carosi[1]

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Since Ruth Harrison's 1964 book "Animal Machines" and its profound impact on public opinion and quality of life of farmed animals, scientific research on non-human animal welfare expanded to other captive settings such as research laboratories, zoos and shelters. Multiple conceptual frameworks have been produced and shared in attempts to define the complex concept of animal welfare, as well as different philosophies and scientific approaches to assess it and improve it. No matter what the emphasis has been put on (e.g., the basic health and biological functioning, a natural living, or the affective states of the animal), the more scientific research has been carried on, the more progressively the concept of welfare as an emergent property of the "animal system" has established itself. As such, welfare may be differently perceived by each individual, as the expression of the combination between its species-specific morpho-anatomical, physiological, and behavioral adaptations, together with its ontogenetic trajectory and experiential life, combining genetics with epigenetics, and species-specific with individual needs. The more recent "Five Domains Model" has prompted scientists to focus on what is now almost universally recognized as the core of an animal welfare state: its mental state. In this talk I will discuss for example the role in animal welfare and key value of: experience and emotions, with emotions teaming up with individual experience and cognition during the process of assessing situational contingencies - preparing the organism for an optimal response - as parts of the evolutionary mechanisms underlying animal decision making; the ability to cope with environmental challenges; having opportunities to exert choice and control over the environment, enhance feelings of autonomy, i.e., 'animal agency'. In the context of animals under human care, animal-centered welfare management should prioritize providing opportunities for comfort-related activities, as they are determinants of animal quality of life - 'a life worth living' – aimed at helping animals not merely to survive, but thrive. Animals under human care too often face a mismatch between their built-in "equipment" (adaptations) and the challenges of their current circumstances, whether wild or domestic species. Emphasis will also be given to the lexicon used in welfare science, of its evocative and often misleading power, since definitions and terms used affect the way animals are treated.

Workshop
Etica, Benessere animale e 3R

Poster

Adaptive evolution and individual ontogenetic trajectory: the biological dimensions of welfare

Monica Carosi[1]

[1] *Department of Sciences, "Roma Tre" University*

Since Ruth Harrison's 1964 book "Animal Machines" and its profound impact on public opinion and quality of life of farmed animals, scientific research on animal welfare expanded to other captive settings such as laboratories and zoos. Multiple conceptual frameworks have been produced and shared in attempt to define the complex concept of animal welfare, as well as different philosophies and scientific approaches to assess it and improve it. No matter what the emphasis was put on (e.g., the basic health and biological functioning, the natural living conditions, or the affective states of the animals), the more scientific research was carried on, the more progressively arose the concept of welfare as an emergent property of the "animal system". As such, welfare may be differently perceived by each individual, as the expression of the combination between its species-specific morpho-anatomical, physiological, and behavioral adaptations, together with its ontogenetic trajectory and experiential life, combining genetics with epigenetics, and species-specific with individual needs. With the more recent "Five Domains Model" scientists were prompted to focus on the core of an animal welfare state, that is its mental state. In this talk I will discuss the importance of experience, of emotions, of opportunities of choice and control over the environment, as determinants on animal welfare and management. In fact, animals in captivity too often face a mismatch between their evolved adaptations and the challenges of their current circumstances. Emphasis will be also given to the lexicon used in the study of welfare, of its evocative and often misleading power.

Joining the Italian Animal Welfare Bodies Network: a simple way to improve animal welfare

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Launched by the National Committee for the Protection of Animals used for Scientific Purposes (CNPA), the Italian Animal Welfare Bodies network (CIO) started in January 2025 and currently comprises 84 AWBs from academic, private, and public research institutions. CIO is supported by a steering committee composed by 9 representatives (3 from North Italy, 3 from Centre Italy and 3 from South Italy) and meets annually with the CNPA focusing on promotion of the 3Rs; providing topics of discussion and clarification to competent authorities; dissemination of good practices; facilitating information exchange to ensure a harmonized approach at the national level; offering guidance on specific topics related to animal welfare.

Here we present the experience of three AWBs from different institutions that have decided to join CIO.

Since 2019 the University of Trieste houses a barrier facility to maintain pathogen-free animals, improve animal well-being and ensure reproducible experimental data. SPF housing conditions with limited access to authorized personnel only make it necessary to improve best practices and procedures for integrating skills, knowledge and competence of all the professional figures involved.

Vetspin performs safety and efficacy studies in different animals for food production, including poultry, rabbits, pigs and cattle. To meet the requirements for all these species it's a challenge, as for every one of them very different installments, feeding, climate conditions, enrichments, and management is necessary due to their different age and weigh. All of them are like a different species with regard to the behavior and the needs to guarantee their safety and well-being.

The IRCCS Santa Lucia Foundation performs basic and translational research studies in the field of neuroscience with the use of laboratory animals, especially transgenic models of disease (eg, models of Alzheimer's, Parkinson's, Muscular Dystrophy, ALS, etc.) with very different management needs. It is therefore necessary to have a broad and varied knowledge of their physiological and behavioural needs. In addition, in keeping with the 3Rs principle, whenever possible, studies are first conducted using transgenic strains of Drosophila melanogaster (Meigen, 1830) before moving on to murine strains.

The recent settlement of the Italian AWBs network demonstrates the perceived need for a coordinated national approach, offering useful insights and practical tools for the affiliated AWBs.

Sessione

Museologia e Comunicazione

comunicazioni orali

The museum anthropological collections within the context of the decolonization practice: challenges and perspectives.

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The anthropological collections housed in many Italian museums encompass a wide range of assets. In addition to human skeletons, these collections are enriched by a large variety of items (anthropometric instruments, ornaments, objects for daily or sacred use, ethnographic materials of various kinds, etc.). Among these, plaster face casts represent particularly unique objects, positioned midway between human remains and artifacts.

Originally produced to illustrate so-called racial differences, most of these casts were made from living individuals during scientific and colonial expeditions. They were not merely trophies, but rather legitimate tools for research and education, capable of conveying human features that could not be fully captured through photographs or measurement tables. As such, they played a relevant role in the development of Physical Anthropology as an independent scientific discipline between the mid-nineteenth and early twentieth centuries.

A considerable number of plaster casts are still held in institutions across Europe, the United States, and Africa. In particular, many Italian scientific museums preserve these assets. The Anthropological Collections of the University of Bologna, for instance, include hundreds of plaster face casts. Among them, the Lidio Cipriani (1892–1962) collection consists of 95 replicas of such casts, realized during the expeditions in Africa and in Asia between 1927 and 1935.

Many of us anthropologists are actively involved in the management, research, and education on the museum collections, which we have inherited from the founders of the discipline. This legacy now presents new challenges regarding the destin and future of this critical and vulnerable heritage, for which we bear great responsibility.

Within this context, the Horizon Europe project COLUMN (Colonial Legacies of Universities: Materialities and New Collaborations) (2025–2029) aims to thoroughly investigate the meanings and future perspectives of these collections, with a particular focus on the plaster face casts housed at the University of Bologna. This is a collaborative project led by Utrecht University, involving other European institutions as well as universities in the Global South (South Africa and Mexico). Here, we present the project's design and initial findings, reflecting on the current role of scientific anthropological museums facing with these new and complex challenges.

The Museum of Sardinian Anthropology and Ethnography and the Museum of Zoology at the University of Cagliari

Antonello Mulas[1], Rita Cannas[1], Mauro Argiolas[1], Federica Deidda Canelles[1], Marco Melis[2], Valeria Succa[1], Vitale Sparacello[1], Elisabetta Marini[1]

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[2] *Università di Cagliari - Dipartimento di Scienze Biomediche*

The Museum of Sardinian Anthropology and Ethnography and the Museum of Zoology, both part of the Interdepartmental Centre for Museums, Collections and Historical Archives at the University of Cagliari, are engaged in activities in the fields of conservation, education, and science communication. While maintaining their distinct disciplinary focuses, the two museums collaborate closely to promote the integration of cultural and natural heritage, and to enhance public engagement and interdisciplinary teaching.

The Museum of Zoology (https://web.unica.it/unica/it/museo_zoologia.page) was founded in the very early 1800s at the behest of the Viceroy of Sardinia, Carlo Felice. Although the main nucleus was established during the 19th century, the acquisition of new specimens has continued up to the present day, so that many of the collections cover a time range of 200 years. The collections now comprise over 10000 specimens of the main animal types, many of them of high scientific, conservation and educational interest. Sardinian fauna is particularly represented, but species from all around the world are present. Among vertebrates, the ornithological and ichthyological parts are the most numerous, with about 2000 specimens. Arthropods, mainly Insects and Arachnids comprise approximatively 7000 specimens. The osteological collection ranges from the few centimeters of the mole skeleton to the over 15 meters of that of the finwhale. Since their inception, the collections have been utilized in research and education, and they continue to have a significant influence on our comprehension of animal biodiversity.

The Museum of Sardinian Anthropology and Ethnography (MUSAE; https://www.unica.it/unica/page/it/musae) was founded in 1953 with the aim of promoting knowledge of the prehistory and history of the Sardinian population through the study of human skeletal remains and evidence of recent cultural traditions. The museum houses and partially exhibits a wide range of materials, including over 10,000 human skeletal remains from prehistoric to modern times, cranial casts illustrating hominid evolution, two mummies, historic anatomical preparations from the last century, and objects from traditional agro-pastoral daily life, including furnishings, tools, musical instruments, clothing, and ex-votos. It also has a collection of more than 20,000 slides and videos. A large sample of skulls has been reconstructed in 3D and will be made freely available for research purposes.

Studying the Italian tardigrade biodiversity through the Citizen science project “Tardigrades go to school!”

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Italy is a biodiversity hotspot in Europe, rich in species, endemic organisms, and diverse biogeographical regions—with tardigrades contributing to this richness. The knowledge of Italian terrestrial tardigrade biodiversity remains incomplete, as many regions are almost unexplored. Due to their distinctive appearance and remarkable stress resistance, tardigrades have captured public interest, especially in younger generations. The Citizen Science project ‘Tardigrades go to school!’ engages students in mapping the distribution of moss-dwelling tardigrades, aiming to develop awareness of biodiversity and a sense of responsibility for its conservation.

Students are introduced to tardigrades and their ecological importance through meetings and educational materials. During hands-on activities at school, they collect moss samples from the garden, extract and observe tardigrades using everyday tools. School trips provide further opportunities to collect mosses, complete data sheets, search for tardigrades, and send samples to tardigrade specialists for faunistic analysis using integrative approach.

Participating classes receive certificates, and findings are shared via reports, public meetings, and the EvoZooLab website. Samples collected from primary and secondary schools in Northern Italy have already revealed a high degree of biodiversity, including rare and new tardigrade species. The project is now expanding to involve more schools throughout Italy, enabling a comprehensive study of tardigrade biodiversity and distribution, as well as monitoring their conservation status. It fosters citizen participation in scientific research and promotes environmental awareness and interest in tardigrades among students.

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Imaginary creatures in the classroom: speculative biology as didactic tool

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Speculative biology is a discipline between art and science, proposing to make physiologically and evolutionarily plausible descriptions of imaginary organisms. Having existed for decades as a niche pastime, it is now increasingly featured in popular entertainment; its subdiscipline concerned with the possibility of life on other planets, known as exobiology or astrobiology, is increasingly treated as a scientific branch worth of study. Since speculative biology requires a solid grasp of the principles of evolutionary biology, but also engages the imagination and creativity of the practitioner, its use as didactic tool suggests itself. Some previous efforts have already taken place, such as asking students to build phylogenies of imaginary organisms or construct fictional but plausible biospheres. We propose a similar exercise in which zoology students (bachelor course in Natural Sciences) are asked to analyse and deconstruct creatures from folklore and mythology, pointing out biologically implausible traits, and then redesign them with a plausible physiology and place them in a plausible evolutionary and ecological context. We present preliminary results of such designs, as well as considerations about the potential of this sort of exercise in helping the students engage critically with the biological principles learned in the classroom, without resorting to rote memorization.

Sessione

Museologia e Comunicazione

poster

The Recovery and Valorization of an Ancient Zoological Collection

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The University Museum of the "G. d'Annunzio" University of Chieti – Pescara has stood out in recent years for its research activities in the fields of anthropology, geo-paleontology, and natural sciences. It has significantly expanded its collections, affirming its role as a "place of memory" and an exhibition space dedicated to the knowledge and dissemination of Natural Sciences as well.

A recent addition to the museum is a new zoological collection, consisting of a large number of animals preserved using various methods (in formalin, dried, or taxidermied), mainly comprising specimens collected and prepared locally in the second half of the 19th century. The collection is the result of a series of donations and bequests from public institutions, such as the "G.B. Vico" High School of Chieti, and private individuals. It also includes specimens prepared in the museum's own laboratories, where disinfestation was carried out on samples—fish, birds, and small mammals—that had been affected by insect larvae.

Following the disinfestation, a cleaning phase was undertaken to remove external pollutants from the birds' plumage, mammals' fur, and keratinized parts (beaks, legs, etc.). The specimens were relabeled in a clear and accessible manner, while still preserving the historical labels, and were newly catalogued.

The portion of the collection currently on public display includes rare specimens and even extinct species, all originating from the Chieti area or, more broadly, the Abruzzo region. Particularly noteworthy are the specimens collected around 1863 by students of the Royal High School of Chieti under the guidance of Professor Florindo Rocchetti (Torrevecchia Teatina, 1820 – Chieti, 1867), a physician who, in 1854, left his profession to dedicate himself to teaching Natural Sciences.

The historical value of this collection—composed largely of passerine birds—is truly exceptional, representing the only and irreplaceable testimony of the local fauna present on the hills of Chieti more than a century and a half ago. Due to all these characteristics, the zoological collection also holds great educational value, but above all, it has significant scientific importance.

The Museum of the Sea and Wetland Areas of Marceddì Comes to Life in Sardinia

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The project "Museum of the Sea and Wetland Areas of Marceddì," promoted by the Municipality of Terralba (OR) with scientific support from the Department of Biology, Ecology and Earth Sciences at the University of Calabria, aims to enhance and showcase the rich environmental, natural, and cultural heritage of the Marceddì wetlands. Funded under Regional Law no. 17/2021, the project involves the setup of an exhibition center in a municipally owned building located at Via Lungomare 49, Marceddì, in the municipality of Terralba (OR).

The exhibition will span two floors and five thematic areas: the lagoon room, the sea stairs, the marine animals room, the bird room, and the Marceddì fishing world. Visitors will be guided through an itinerary that explores marine, lagoon, and coastal environments, with a special focus on the interaction between nature and human activities.

The exhibition spaces will include display cases, life-sized dioramas, informational panels, aquariums, terrariums, and interactive multimedia totems. The tour will be enriched with digital content such as drone-filmed videos, multimedia guides on flora and fauna, educational games for children, and teaching materials for schools, supporting both hands-on and learning activities.

Outside, the museum will feature an institutional sign and a traditional fishing boat, an iconic symbol of the local community. The project also includes public and scientific outreach events to promote civic engagement, environmental awareness, and local identity. This initiative stands out as one of the few museums in Italy, and the only one in Sardinia, specifically dedicated to wetland ecosystems.

Soil Meiofauna Advanced Taxonomy School – SoilMATs: Advancing Taxonomic Knowledge Through Training

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Recent applications of integrative approaches in taxonomy have accelerated species descriptions; however, much remains to be done to understand the diversity and distribution of meiofauna in soil environments, primarily due to the taxonomic impediment. To address this gap, the SoilMATs project aims to train a new generation of taxonomists, also selected through an open European call, by combining online and hands-on activities to equip 25 young researchers with specialized knowledge in Tardigrada, Rotifera, and Nematoda. The large number of applications (84 for 20 open positions) highlights the strong demand for advanced training in taxonomy, even for seemingly "neglected" taxa. Additionally, the program's secondary goal is to enhance soil meiofaunal knowledge in three target protected areas across Europe through teaching.

The first field training and sampling took place in two natural protected areas in the Northern Apennines: Rocca Malatina Park and Panaro River Park (Italy). The demonstrative analyses conducted during the training focused on taxa identification, integrating both morphological (LM, SEM, and CLSM) and molecular (DNA barcode) approaches.

Sampling during the training contributed to a broader understanding of the biodiversity within the sampled protected areas. For instance, a rare tardigrade species lacking claws was discovered in a conspicuous population, in a previously unsampled moist riverbed sediment. The specimens were assigned to the known species Apodibius confusus through morphological analyses and the first DNA barcode sequence for the taxa were produced. Furthermore, advanced microscopy techniques, showed during the training, revealed the complete absence of claws as a structural trait, with no associated vestigial characters.

The training activities accelerated faunistic analysis, facilitated by the number of students, which allowed for more effective and faster sampling, sorting, and taxonomic work. Beyond direct training, the project's multi-local approach is expected to have a wider impact by, for instance, enhancing faunistic studies and identification of new taxa, as demonstrated in the given example.

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The digitisation of the University of Bologna's "Collezioni di Antropologia": a virtual exhibition through innovation, research and conservation

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In the context of the increasingly widespread use and applications of digital tools in museums to enhance accessibility and the conservation of cultural heritage, this ongoing project aims to create a virtual exhibition of the University of Bologna's "Collezioni di Antropologia". The first nucleus of these collections dates back to 1908, when Professor Fabio Frassetto established the first chair of Anthropology at the University of Bologna. Over the subsequent years and up to now, various items have enriched the collections, particularly elements from the Documented Human Osteological Collections (DHOC) of the University of Bologna. This project involves scanning selected anthropological items within the museum halls of the "Collezioni di Antropologia" using an ARTEC Space Spider 3D structured light laser scanner. These digitised selected anthropological items will form the core of the virtual exhibition, which will be divided into three thematic sections, mirroring the physical tour of the museum. The first section will focus on the origin of Anthropology as a discipline in Italy, featuring a selection of anthropometric and osteometric instruments, along with the famous casts from the Cipriani collection. The second section will explore human evolutionary history, focusing on some of the most significant fossil hominins, such as the Taung Child (A. africanus), especially relevant this year as it marks the centenary of its first publication in Nature. The third section will be dedicated to skeletal biology, bioarchaeology and paleopathology. The skeletal biology section will help virtual visitors understand which human skeletal traits are important for identifying sexual dimorphism and patterns of bone growth. The bioarchaeology will connect anthropological knowledge with cultural practices, illustrated by examples of cranial deformation. At the same time, some individuals will be digitised to showcase pathognomonic traits of skeletal pathologies. Finally, a dedicated virtual exhibition space will be developed to present these elements in an engaging and informative way, supporting a deeper understanding of the collections and their digital presentation. The online experience—enhanced with accessible content and interactive tools—aims to boost public engagement, make scientific knowledge more widely available, and support museum operators in communication, outreach, and heritage conservation of these sensitive cultural assets.

The bird collection of the 'Museo di Zoologia' at the University of Cagliari: past and present importance

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The ornithological collection of the Museum of Zoology at the University of Cagliari (Sardinia, Italy) has been recently re-organized and revised. The collection mainly comprises specimens representing Sardinian fauna that were most likely collected on the island. However, it also contains a significant number of exotic, land and sea birds from different parts of the world. It currently hosts more than 1000 mounted specimens, tens of skins and a few skeletons with representatives of 30 orders, 111 families and 465 species.

The original nucleus was established in 1802 by order of Carlo Felice, Viceroy of Sardinia, who later donated it to the then Royal University of Cagliari. This historical collection had its greatest development in the first half of the 19th century, especially during the period 1840-1858, in which Gaetano Cara was director of the Museum. Although to a lesser extent, the acquisition of new finds has continued up to the present day.

The most complete section is represented by the birds of prey, for which there are several specimens of all the species historically present in Sardinia, including those now extinct on the island, such as the Bonelli's Eagle (*Aquila fasciata*), the White-tailed Eagle (*Haliaeetus albicilla*), the Cinereous Vulture (*Aegypius monachus*) and the Bearded Vulture (*Gypaetus barbatus*). Similarly, other taxa, extinct in the Island, are present, such as the White-headed Duck (*Oxyura leucocephala*), and the Black Wheatear (Oenanthe leucura). The collection includes also some rare specimens, exceptionally captured in Sardinia: Little Bunting (*Emberiza pusilla*), Rosy Starling (*Pastor roseus*), and Cream-coloured Courser (*Cursorius cursor*). Among the 'exotic birds' numerous species of hummingbirds and sunbirds certainly stand out, as well as the specimens of highly threatened species, such as the Yellow-crested Cockatoo (*Cacatua sulphurea*) or the Tristan Albatross (*Diomedea dabbenena*) or even globally extinct species such as the Passenger Pigeon (*Ectopistes migratorius*) and the Slender-billed curlew (*Numenius tenuirostris*).

The historical collection has still an undoubtable importance, not only to scientists but also to pupils and amateurs. It can help to understanding the distribution and composition of the ornithofauna in Sardinia and its changes through time, to educate children and sensibilize to public as well as it can provide irreplaceable information to protect our stunning bird biodiversity.

“INCONSAPEVOLI INVASORI”: increasing awareness on biodiversity loss

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The Natural History Museum of the University of Pisa designed and installed a temporary exhibition entitled “Inconsapevoli invasori. Le specie aliene nelle acque interne e le minacce alla biodiversità” from October 2024 to January 2025. Theme of the exhibition was the invasion of alien species in Italian freshwater environments, with a focus on their impact on biodiversity loss and on the causes of the invasions.

Live animals were displayed in aquaria and aqua terraria, for a total of 15 species including fish (6 species), invertebrates (2 species) and freshwater turtles (6 exotic species and the native European pond turtle, *Emys orbicularis*). We selected both highly invasive species (e.g. *Trachemys scripta*, *Procambarus clarkii*) and other species representative of the main impacts of aliens: direct competition, predation, hybridization, transmission of pathogens. We included also species arrived more recently on the national territory (e.g. *Pseudemys* sp.), to show how this threat is still ongoing and developing.

The use of live animals was aimed at maximizing public involvement, promoting better transmission of the scientific concepts of the exhibition. All the individuals on display came from Tuscany’s natural or semi-natural environments, with the purpose of helping the visitors to better understand how the problem of alien species is rooted in every territory, including their own. The exhibition panels included general information on the topic, as well as updated scientific data on the quantity of invasive species in Italy, economic damage and problems related to the spread of aliens; there were also descriptive sheets of the non-native species on display and a section dedicated to the Life URCA ProEmys project on the European pond turtle, of which the Museum is a partner.

The exhibition was integrated with a multimedia display of photos and videos of alien species specially created by professionals, expert naturalists and photographers of national relevance.

To raise awareness among visitors of the various topics covered in the exhibition, we organized three events held by various experts, in line with the objectives of Agenda 2030. These dissemination activities aimed at increasing public awareness about the impact of alien species on biodiversity loss and provided indications on the correct behaviors to be held in case of purchase or discovery of these species in the wild.

The heterobranch Corner Project at the Unisalento Marine Biology Museum ”Pietro Parenzan” in Porto Cesareo (LE)

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In the XXI century, advancements in technology and the growing abundance of information have led to significant changes in communication strategies, aiming to capture people’s attention and facilitate their understanding of scientific topics.

In this framework, we are going to combine a traditional exhibition method (the exhibition panel) with the use of soft toys to increase the attractiveness of the scientific and informative proposal, especially towards a younger audience. Multimedia content can be accessed via QR codes placed in the different sections, in order to foster a user-friendly and accessible experience and an in-depth comprehension of scientific concepts.

The main objective of the “Heterobranch corner” project at the UniSalento Marine Biology Museum “Pietro Parenzan” in Porto Cesareo (Lecce), is to provide everyone with the chance to discover the Mediterranean heterobranch diversity, a group of specialized gastropod molluscs to foster knowledge and inspire a heightened sense of appreciation and concern for their protection and conservation.

In order to reach a wide-ranging audience, the “Pietro Parenzan” Marine Biology Museum was chosen as the ideal location to install these panels: recently renovated and re-opened in December 2024, it has high visitor footfall, particularly comprising of students who visit the museum during the school year and families who go there during the summer.

The corner consists of a 2m by 2m panel and a 2m by 1m panel, inside which there are photographs dedicated to nudibranchs, sacoglossans, anaspids and cephalaspids, chosen as representatives of the extreme variability of shapes and colors present within the subclass of heterobranchs. There are also insights into their life cycle, defense strategies and how to find and describe a new species; each section features QR codes that link to videos and multimedia storytelling created specifically for the purpose. A plexiglass cube houses the soft toys of two nudibranchs, each about 40 cm large.

In a time when the protection of the sea has a fundamental role and conservation actions are increasingly urgent, to promote knowledge of the sea fauna becomes an essential task of marine biology museums to widen ocean literacy among the civil society.

Sessione
Tema libero

Poster

First record and biological analysis of *Pyroteuthis margaritifera* in Sardinian waters

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The jewel enope squid, *Pyroteuthis margaritifera* (Rüppell, 1844), is the only known representative of its genus in the Mediterranean Sea. Specimens are rarely encountered, and to date, existing records have merely confirmed its presence in the region. Throughout its entire distribution range, only few biological data are available mainly focused on the comparative morphology of its distinctive photophores located on the tentacles, eyes, anus, gills, and abdomen. In June 2016, for the first time in Sardinian waters, a specimen identified through molecular analysis as *Pyroteuthis margaritifera* was accidentally captured by a trawl net at a depth of 250 meters. The individual, which measured 42 mm in dorsal mantle length and weighed 11.3 g, exhibited all the photophores previously described for the species except those on the tentacles. This absence contrasts with observations from six other Mediterranean specimens (from the Strait of Messina) recovered from the stomach contents of Thunnus thynnus, in which tentacular photophores were clearly present. Biological analysis of the Sardinian specimen revealed that it was a mature male, containing 372 spermatophores inside the Needham's sac, with a mean total length of 8.62 mm. Meristic data were recorded on the hooks present on the tentacle club and on the fourth arms, and the main measures of the beak were also recorded. Overall, the morphometric and meristic data reported herein represent the first comprehensive biological information and anatomical documentation available in Mediterranean for this elusive species. The absence of tentacular photophores in this specimen, in contrast with what reported in the literature, highlights the need for further studies on additional Mediterranean specimens using an integrative approach that combines morphological and genetic analyses.

How microhabitat characteristics influence the females' nest site selection of the invasive alien species *Trachemys scripta* in the Angitola Lake (Calabria, Southern Italy)

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Invasive alien species (IAS) represent a major threat to global biodiversity, ecosystem functionality, and native species, with particularly pronounced impacts in protected areas. The red-eared slider turtle (*Trachemys scripta elegans*) is recognized as one of the 100 most damaging IAS worldwide due to its adaptability and widespread distribution. This research focused on the nesting ecology of *T. scripta* within Angitola Lake, a significant Ramsar site and WWF Oasis located in Calabria, Southern Italy. The primary objectives were to gain a deeper understanding of its reproductive strategies in this environment and to provide crucial information to guide the development of effective management interventions.

The study was carried out during the active nesting season of 2007, spanning from April to October. Through field surveys, three primary reproductive zones and three secondary sites along the lake's shoreline were identified. A total of 309 natural predated nests were documented across these locations. At each recorded nest, four measurements of the nest microhabitat were taken: the distance to the shoreline, nest depth, vegetative cover, and soil characteristics. Univariate analysis and a Generalized Linear mixed model (GLMM) were employed to investigate the nest site preferences of the females.

The findings revealed a clear preference for mixed habitats characterized by soil combinations such as dirt/sand and dirt/clay, suggesting these conditions offer optimal characteristics for oviposition. Nesting activity was predominantly concentrated within the first 30 meters from the shoreline. Notably, habitat and soil selection appeared to shift throughout the nesting season, potentially in response to changing environmental factors, including water availability. However, the results of the generalized linear mixed model (GLMM) indicated that only the distance from the shoreline was significantly influenced by vegetation type (with greater distances observed in reforested sites). The ecological plasticity exhibited by *T. scripta*, enabling it to exploit a broad range of nesting microhabitats, represents a significant ecological threat to local biodiversity. This study provides detailed insights into the reproductive ecology of this invasive species, underscoring the need for the implementation of integrated management strategies aimed at mitigating its ecological impacts.

Insights into the solitary ascidian *Ciona robusta*: a zoological model to study innate immunity

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Ascidians (Tunicata) are important models for studying the innate immune system due to their evolutionary proximity to vertebrates. As sessile filter feeders, they are exposed to environmental stressors, including pathogens, requiring a strong immune response. *Ciona robusta*, a solitary ascidian found in temperate marine environments, is a widely used model in zoological research, known for its well-characterized immune system, transparent body, and simple anatomy. This species has distinctive morphological features, such as a thick outer tunic for water filtration and a prominent pharyngeal basket, where immune cells play a key role in pathogen defense.

This study investigates the immune responses of *C. robusta* under bacterial challenge, specifically focusing on the effects of lipopolysaccharide (LPS) exposure. Using immunohistochemistry and enzyme assays, we observed activation of key immune pathways, including Toll-like receptor (TLR) and nuclear factor kappa B (NFB) signaling, central to the inflammatory response in both invertebrates and vertebrates. These immune molecules were significantly upregulated in the pharyngeal tissues 4 hours post-LPS injection. Inflammatory nodules were found in the lumen of pharyngeal vessels, with endothelial cells actively participating in the inflammatory process. Histological analysis of untreated and buffer-injected ascidians confirmed the positive immune reactions. Enzymatic assays revealed a marked increase in the activity of immune-related enzymes, such as phenoloxidase, glutathione peroxidase, lysozyme, alkaline phosphatase, and esterase, following LPS exposure. These enzymes, involved in pathogen recognition, oxidative stress response, and antimicrobial activity, play critical roles in mediating the inflammatory response.

Our findings underscore a complex, multi-faceted innate immune activation in *C. robusta*, resembling vertebrate immune processes, and provide valuable insights into the molecular and cellular dynamics of pathogen defense. These results suggest that ascidians may serve as an excellent model organisms, offering opportunities to explore universal principles of immune regulation from tunicates to vertebrates. Future research should focus on bridging knowledge gaps and further elucidating the evolutionary conservation of immune mechanisms across species.

Loss of meiofaunal biodiversity under thermal stress regime: the vulnerable seagrass-bivalves association

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Biological positive interactions are key drivers in shaping the biosphere, yet climate change can alter these relationships with consequences for biodiversity. In the marine realm, a notable example is the mutualism between lucinid bivalves and seagrasses, both acting as ecosystem engineers. Despite their ecological relevance, the response of such interaction and associated benthic biodiversity to extreme climatic events remains poorly understood. In this study we investigated how meiofaunal biodiversity and trophic status change in the presence or absence of the association between the seagrass *Cymodocea nodosa* and the lucinid *Loripes orbiculatus* under simulated marine heatwaves (MHWs). A 17-day mesocosm experiment was conducted in laboratory, including four conditions: bare sediment, sediment with *C. nodosa*, with *L. orbiculatus*, and with the association. A MHW was simulated by gradually increasing water temperature to +5°C above in-situ one, then maintaining it for 10 days, while control mesocosms remained at 25°C. The highest meiofaunal richness (8 taxa) was found in the *C. nodosa* condition, followed by the seagrass-lucinids association (7 taxa). After the MHW, all conditions showed a decline in taxa richness, with the greatest loss (43%) found in the plant-bivalve condition where we report the extinction of Copepoda, Polychaeta and Oligochaeta. At the end of the MHW, taxonomic composition significantly varied with a decrease in the abundance of heat-sensitive taxa (e.g., copepods and foraminifera) and an increase of more tolerant ones (e.g., nematodes). In parallel, the quantity and nutritional quality of sedimentary biopolymeric carbon declined across all impacted conditions, especially in the seagrass-lucinids one. Our results suggest that the ecosystem characterized by the association between *C. nodosa* and *L. orbiculatus* seemed to be the most sensitive one among those tested to extreme ocean warming events, showing the greatest loss of meiofaunal biodiversity and available resources after MHWs. This finding contributes to our understanding of how disturbances may impact biodiversity and reduce the resilience of ecosystems especially those with complex interactions.

Multimodal communication in ants: the interaction between chemical and vibro-acoustic signals in three Myrmicinae species

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Communication is a fundamental aspect of animal behaviour, with signals playing essential roles in courtship, foraging, and predator-prey interactions. In social insects like ants, chemical communication prevails, guiding a wide range of collective behaviours. However, some ant species use a stridulatory organ to produce vibro-acoustic signals that can trigger a behavioural response, either independently or in conjunction with chemical cues. Despite the widespread occurrence of stridulation in ants, its biological significance and interaction with other signalling modalities remain poorly understood. In this study, we explored the interplay between chemical and vibro-acoustic signals in the context of alarm communication in three Myrmicinae ant species: *Crematogaster scutellaris*, *Myrmica scabrinodis*, and *Manica rubida*. We used Laser Doppler Vibrometry and Stir Bar Sorptive Extraction (SBSE) to characterize vibrational signals and volatile pheromones produced by restrained ants. We designed an artificial arena to investigate workers' behavioural responses to pheromones and vibrations separately, as well as in combination. Our findings confirm chemicals and vibro-acoustic signals to be specie-specific. Furthermore, we found different response to multimodality in different species. In particular, chemical and vibro-acoustic signals are both necessary to trigger an attractive response in *C. scutellaris* and *M. rubida*, while only chemicals are sufficient to attract nestmates in M. scabrinodis. Interestingly, in *M. scabrinodis*, the addition of a concomitant stridulatory signal disrupts this attraction. These findings suggest the presence of modulatory effects that are potentially subject to environmental constraints. In particular, the differences in the active space of each signal component can result in varying responses depending on the environment or distance from the source. Furthermore, the three species occupy distinct microhabitats (*C. scutellaris* is primarily arboreal, *M. rubida* inhabits open high-altitude areas, and *M. scabrinodis* is a generalist) which may influence how chemical and vibrational signals propagate, ultimately affecting receiver perception. Overall, our results reveal species-specific response to multimodality in species that inhabit different micro-habitats, providing insights into potential selective pressures and constraints shaping the evolution of multimodality in complex social systems.

Peripheral sensory learning and "Taxonomic Behavior" in *Octopus vulgaris* arms

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Octopus vulgaris serves as an excellent model organism for the study of motor control and sensorimotor integration due to its highly specialized nervous system and advanced manipulative abilities. Each arm possesses a remarkable degree of functional autonomy, supported by a decentralized neural architecture in which over two-thirds of the neurons are located in peripheral ganglia. This organization enables the execution of complex movements without needing continuous central control. This decentralized neural organization, combined with a highly flexible morphology and refined manipulation skills, enables the octopus to explore and interact with its environment in a highly adaptable manner.

In this study, we present a novel experimental protocol developed for the controlled analysis of sensory systems at the level of individual arms. This protocol maintains the ability to monitor motor patterns accurately and assess taxonomic behavior in terms of species-specific behaviors. The protocol includes a progressive training sequence: the pre-training phase, in which the animal is trained to interact with a device containing a single real food stimulus, and the experimental phase, in which it is required to discriminate between two 3D-printed artificial anchovies and one real anchovy. The goal is to condition the animal to associate specific interactions with a food reward. For this protocol, a transparent plexiglass device was developed, consisting of three independent compartments, each equipped with an opening for arm insertion and a removable lid. A dedicated "ethological photo set" was also assembled for behavioral data collection, enabling detailed analysis using the BORIS software. *O. vulgaris* demonstrates the ability to distinguish between real and artificial stimuli using tactile/chemical and visual cues. Through the training phases, octopuses exhibit learning behaviors, such as associating specific tactile/chemical and visual stimuli with food rewards. This would be consistent with documented evidence of their ability to learn through conditioning and retain information over time and exhibit taxonomic behavior. Individual variability in response to stimuli could indicate behavioral plasticity and personality-like traits, suggesting that *O. vulgaris* can adapt its exploratory strategies based on past experiences and environmental changes.

Preliminary analysis of benthic fauna associated with vermetid reef along the Ionian coast of Apulia (Southern Italy)

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Vermetid reefs are considered very important ecosystems, due to their role in enhancing habitat complexity, modulating coastal morphological processes and providing shelter and food resources for many invertebrates. This study aims to investigate for the first time the benthic associated fauna with vermetid bioconstructions newly reported along the Apulian coast of the Ionian Sea, in the province of Taranto. The research area was located along 40 km of coastline, between the localities of San Vito and Campomarino (TA). Three study sites were selected and, at each site, two quantitative samplings, using squares 10 × 10 cm, were carried out along three transects by scraping off thin vermetid encrustation. Samples were sorted and preserved in 70% ethanol for species identification and counting.

The present study reports a rich and diverse fauna associated with vermetid bioconstruction, comprising 3266 individuals across 63 different taxa and 6 Phyla (Porifera, Nemertea, Mollusca, Annelida, Arthropoda, Chordata). Amphipods were found to be the most abundant, followed by Bivalves and Polychaetes. Some species (e.g., *Perinereis cultrifera*, *Syllis amica*, *Mytilaster minimus*, *Caprella grandimana*) were common across all three sites, representing typically species found in other Mediterranean vermetid reefs. Among the identified taxa, alien species such as Pseudonereis anomala and Mesanthura romulea were also detected. Notably, the high number of new crustacean species has never been reported in association with vermetids, partially addressing the knowledge gap for this taxonomic group.

The comparison with the benthic fauna associated with the vermetid bioconstruction newly described along the Adriatic coast of Apulia, revealed that only 27% of the species were shared, highlighting the unique species composition of each vermetid formation depending on the geographic location. This finding has important conservation implications, as it suggests that each vermetid bioconstruction supports site-specific biodiversity, underscoring the ecological value of vermetid bioconstructions in the coastal areas. Therefore, it is crucial to integrate these habitats into regional and national conservation policies to ensure the protection of their unique associated biodiversity.

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New methodology for monitoring fishery-target species in Marine Protected Areas

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Marine Protected Areas (MPAs) represent ideal contexts for the application of experimental approaches and co-management models, facilitating the reduction of conflicts between the Managing Authority and local stakeholders, and promoting more effective and participatory governance. Among the most relevant activities in terms of biological impact and management interest is small-scale fishing, which represents an extensive pressure on target species and coastal habitats. This study focuses on the MPA "Penisola del Sinis–Isola di Mal di Ventre" (central-western Sardinia). Over the years, numerous monitoring campaigns of fishing activities have been conducted, but the reliability of the self-reported information by fishermen through logbooks has raised concerns about data accuracy. In 2023, to improve the spatial resolution and traceability of fishing activities, GPS geolocation systems were installed on a voluntary sample of nine vessels. Between 2023 and 2024, a total of 317 fishing trips were recorded over 130 days (an average of 1.5 ± 0.6 trips per day). Of the GPS tracks collected, 61% were complete and usable for analysis. Data collected from 2018 to 2025—via logbooks, onboard observations, and visual inspection of landings—enabled in-depth analysis of fishing dynamics within the MPA. These efforts contributed to a more detailed understanding of fishing patterns and behaviors. The comparison between the two data sources showed a match in 60% of cases (118 trips), indicating a fair level of reliability in the self-reported data, while also highlighting areas for improvement in field data collection and validation.

The integrated analysis of GPS tracks and catch records allows for the correlation of fishing trajectories with benthic habitats and zones of varying biological vulnerability, contributing to the understanding of the spatial ecology of fishing effort and its implications for fish communities. The technological advancement of these tools, combined with the expansion of their functionalities, makes them particularly effective in promoting constructive dialogue between MPAs and fishermen, fostering stronger, more transparent collaboration based on objective data.

The use of the otolith reading technique to determine the age of silver eels in Sardinia, Italy

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The European eel *Anguilla anguilla* (Linnaeus, 1758) inhabits rivers and lagoons during its continental growth phase, where the silvering process, essential for reproductive migration, occurs. The timing of silvering is influenced by factors like latitude, environmental conditions, and food availability. This study investigates the metamorphosis to the reproductive stage within the Sardinian lagoon environment where eels are traditionally fished, focusing on the age by otolith analysis, size, and sex at the onset of silvering.

In 2019, 229 eel specimens were collected from two Sardinian lagoons: 109 from Porto Pino (495 ha, southern Sardinia), and 120 from Sa Praia (97 ha, eastern Sardinia). For each specimen parameters as livery, sex, total length, pectoral fin length, eye diameter, ocular index (OI), total weight, and gonad weight were recorded. For age determination, otoliths were processed using a grinding and polishing technique and stained with toluidine blue to enhance the visibility of annual growth rings. The age of each specimen was subsequently correlated with its biometric values and indices.

Out of 229 examined eels, 150 were yellow eels. Among these, 109 were females, with length range 26.0-72.8 cm and weight range 24.5-326.3 g. The remaining 41 were males, with length range 30.5-43.1 cm and weight range 33.8-170.0 g. The other 79/229 were silver eels. Of these, 41 were females, with length range 50.2-72.7 cm and weight range 191.6-778.5 g. The remaining 38 were silver males, with length range 34.0-45.6 cm and weight range 65.0-142.7 g. Female yellow eels age ranged between 2 and 10 years (mean: 4.47±1.36), while male yellow eels age ranged between 2 and 7 years (mean: 4.37±1.22). Silvering females age ranged between 6 and 12 years (mean: 8.49±1.50), while silvering males age ranged between 3 and 7 years (mean: 5.21±1.02). The OI was correlated with the transition from the yellow to the silver eel stage. For females, OI values exceeding 7.40 were consistently associated with the migrant stage. For males, this threshold was at 5.33.

These values are relatively low compared to those observed in continental Europe, which aligns with the evidence that maturation age increases with increasing latitude. Given that *A. anguilla* is both a commercially important species and critically endangered according to the IUCN, this information can be valuable for guiding conservation efforts related to both fisheries management and environmental protection.

A polychaete under pressure: *Sabella spallanzanii* as a zoological model for marine invertebrate immunity and environmental stress response

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The Mediterranean polychaete *Sabella spallanzanii* is emerging as a zoologically relevant model for studying innate immune mechanisms and stress responses in marine invertebrates. Its ecological niche, phylogenetic placement within Lophotrochozoa, and the presence of a mucus-based immune system make it a valuable organism for investigating interactions between environmental pollutants and invertebrate immunity.

Recent studies have focused on the response of *S. spallanzanii* to various stressors, including inorganic arsenic, copper sulphate, methylmercury, and bacterial infection (*Escherichia coli*). Mucus secreted by this species contains a calcium-dependent galactose-binding lectin (SsGBL), with specific agglutinating activity toward Gram-negative bacteria. This component plays a key role in non-self recognition and first-line immune defense.

Following arsenic exposure, which is naturally bioaccumulated in tissues possibly as an antipredatory strategy, the agglutination capacity of mucus lectins was significantly reduced, suggesting a trade-off between chemical defense and immune functionality. Seasonal variations in biological activity were observed, but arsenic tolerance seemed consistent across conditions, pointing toward possible evolutionary adaptation.

Additional experiments investigating the effects of copper sulphate and methylmercury, both alone and in combination with *E. coli* infection, revealed modulation of several immune markers, including esterase, alkaline phosphatase, glutathione peroxidase, lysozyme, and haemagglutination activity. Notably, co-exposure to bacterial and chemical stressors led to suppressed immune responses and altered protein expression profiles.

These findings support the use of *S. spallanzanii* as both a bioindicator in marine biomonitoring and a model system for understanding invertebrate immunity. Its complex immunophysiological responses to natural and anthropogenic pressures provide valuable insights into the evolution of immune strategies and resilience mechanisms in benthic marine organisms.

Terrestrial arthropods as indicators of restoration success in an active limestone quarry

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Limestone quarries are industrial sites that impact natural areas, thus their restoration is crucial for mitigating these impacts and reducing biodiversity loss. Restoration can be achieved through active management projects, such as hydroseeding and soil amelioration. Our study aims to evaluate the results of an active restoration project performed at a limestone quarry in Piedmont (NW Italy) using terrestrial arthropods as ecological indicators. We sampled four areas of the quarry restored in different periods between 1999 and 2016, following the quarry development, and showing different stages of vegetation succession, from sparse grassy vegetation to woodland. Additionally, we included a semi-natural xerothermic grassland outside the quarry as a control area. Arthropods were sampled at 20 points (four for each successional stage, and four at the control site), each consisting of three pitfall traps, filled with 100 ml of 20% propylene glycol as preservative liquid and spaced about 3 metres apart. All traps had been activated for seven days each month, from May to October 2024, for a total of five sampling sessions. Overall, we collected 13164 individuals belonging to 107 families. RDAs and GLMMs were applied to test for differences in community composition and biodiversity indexes according to the restoration stage, and between the quarry and the control area. RDAs showed that the community composition was mostly similar at the different restoration stages and in the control area. Some coprophagous beetle families, such as Scarabeidae and Geotrupidae, were associated with the semi-natural xerothermic grassland, while predators such as Carabidae and several spider families were associated to the earlier successional stages created by the quarrying activity. GLMMs revealed a higher arthropod diversity in the oldest successional stage, now colonized by broadleaved woodlands. On the other hand, species richness reached a higher value in the control area, outside the quarry. These results suggest that predator arthropods can thrive in restored quarries, leveraging on sparse grassy vegetation in the newly restored areas of the quarry. On the contrary, coprophagous and necrophagous beetles are more abundant outside the quarry. Notably, high arthropod diversity in the oldest part of the quarry suggests that active restoration management is a profitable tool for restoring these post-industrial sites and achieving biodiversity conservation targets.

First observations on the electrosensory system of the common smooth-hound shark, *Mustelus mustelus* (Linnaeus, 1758), from the Mediterranean Sea

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The common smooth-hound *Mustelus mustelus* (Linnaeus,1758) is a demersal shark widely distributed in temperate and subtropical coastal waters, including the Mediterranea Sea. Like other cartilaginous fish, it possesses a specialized electrosensory system: the ampullae of Lorenzini (AoLs). These structures, functionally adapted to detect minute electric fields, consist of subdermal sensory organs connected to the environment via canals ending in pores. The morphology, histology and spatial distribution of AoLs vary among species.

This study provides the first description of the gross anatomy and histomorphology of AoLs in *M. mustelus*, aiming to clarify their structure and infer possible ecological adaptation.

Specimens were collected as by-catch from artisanal trammel net fishery in the Egadi Archipelago. Following deep anaesthesia with MS-222 Sandoz, AoLs samples were fixed in paraformaldehyde and processed for macroscopic and histological analysis. Cephalic distribution and gross anatomy were examined macroscopically, while histological sections were stained with Alcian blue and Milligan's trichrome to reveal cellular features.

AoLs are organized into distinct clusters (supraorbital, buccal, hyoid, and mandibular clusters), each composed of a canal and a dilated ampullary portion (AP), both filled with gel and surrounded by connective tissue. Each cluster comprises at least five ampullae, connected to an afferent nerve. Nerve fibre bundles branch at the base of each AoL, enabling separate innervation.

Histological analysis confirmed the general organization of the AoLs, which are primarly located in the rostral cephalic region. The canal is lined with simple squamous epithelium, while the AP is composed of simple columnar epithelium containing supporting and sensory cells. Each AP includes eight alveolar sensory chambers, which are proximally connected to afferent nerves. Nerve bundles reach the base of each AP beneath the central cup, with terminals extending to the base of the sensory cells only.

Our findings seem to highlight a high density of cephalic AoLs and pronounced alveolar subdivision in *M. mustelus*, suggesting enhanced electrosensory reception and signal processing. These characteristics may be linked to specific ecological traits, encouraging future studies on the evolutionary drivers and ecological implications of AoL system in this species.

Monitoring of eel ladder on the Casteldoria dam in the Coghinas River (northern Sardinia, Italy)

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The European eel *Anguilla anguilla* (Linnaeus, 1758), a catadromous species classified as “Critically Endangered” on the IUCN Red List, faces several anthropogenic pressures that have led to stock progressive decline since the late 1980s. One of the most severe ecological problems, beyond the direct effects of adult fishing and the capture of juveniles for aquaculture, is habitat loss due to the fragmentation of watercourses by weirs and dams.

This study describes the monitoring of the eel ladder installed at the Casteldoria dam (Sardinia, Italy), located along the Coghinas River, 15 km upstream from its mouth. The dam is used for power generation by Enel Green Power and the water is also used for drinking and irrigation purposes by ENAS and Consorzio bonifica of North Sardinia. The eel ladder is a total of 37 meters long with an average slope of 63%. It operates by means of water flowing down a covered canal with plastic bristle substrate, which allows eels to ascend upstream. Data for this study were collected through weekly sampling from fall 2022 to fall 2024. For each monitoring day, the total weight of eels was recorded, and biometric analyses of length and weight were performed on a subsample of 30 individuals.

During the reference period, a total of 8,754 eels were counted, with a total weight of 18,612 g. The highest number of individuals counted was 4,814 in winter 2022–2023, while the lowest number was 5 individuals in autumn 2024. The biometric values recorded from the eel subsample across the different seasons were: mean length ranged from 10.24 ± 0.32 cm in autumn 2023 to 17.50 ± 0.71 cm in autumn 2024, while mean weight varied from 1.03 ± 0.58 g in autumn 2022 to 5.47 ± 6.42 g in winter 2023–2024.

The eel ladder is of vital importance to ensure the natural and complete colonization of eels along the river’s main channel. The installed ladder has proven effective in allowing the transit of elvers and yellow eels. No glass eels were detected, likely due to the ladder’s 63% slope, which is probably too steep for this stage. Restoring river continuity, even with structures like eel ladder, is fundamentally important to protect the species and enable its presence even in habitats impacted by human activity. Adopting measures of this kind is definitely a strategy to complement the regulation of the direct exploitation of the species.

Integrative taxonomy unravels the origin and introduction pathway of non-indigenous *Ceratonereis* in the Mediterranean Sea

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The Mediterranean Sea is one of the regions most affected by biological invasions, especially after the opening of the Suez Canal determining the arrival of Info-Pacific species from the Red Sea. Ports and marinas are important gateways for alien species, fuelling the spread of lessepsian migrants that raise concerns due to their impact on local ecosystems. Although polychaetes are among the most abundant taxa in marine benthic communities, genetic data and critical taxonomic revisions are still lacking for many groups compared to other invertebrate taxa, which hinders proper identification of non-indigenous species (NIS). With the aim of tracing the distribution and invasion routes of polychaete NIS in the Mediterranean, this study focused on *Ceratonereis mirablis*, a species originally described for the tropical western Atlantic and reported as a NIS in the eastern Mediterranean. Specimens of *C. mirabilis* from Lebanon and Cyprus were compared with material from the Arabian Gulf, Oman and the Caribbean using an integrative approach combining molecular, morphological and ecological data. Genetic and morphological data highlight the presence of two distinct *Ceratonereis* species in the Mediterranean, one of which occurs on vegetated hard bottoms, while the other in soft sediments only. Even though these lineages were historically referred to as *C. mirabilis*, molecular data show a clear distinction from specimens collected in the western Atlantic. Conversely, the Mediterranean lineages also occur in the Red Sea, which suggests a lessepsian origin of both species and that *C. mirabilis* as currently identified represents a species complex, emphasising the need for a critical revision of this genus.

The arthropod communities of the *Posidonia oceanica* banquette of Sant'Agostino (Latium, Central Italy)

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Banquettes are an important coastal ecosystem formed from the accumulation on the coasts of plant biomass, represented by the dead leaves of the marine phanerogamous plant *Posidonia oceanica* (L.) Delile, and many species of invertebrates and small vertebrates are present within them. These environments are still poorly studied from an ecological and faunistic point of view and are increasingly at risk due to human activities. In Latium, at the locality of Sant'Agostino, near Civitavecchia (Rome), there is one of the most well-preserved banquettes in the Region, which is part of the protected area “La Frasca” Natural Monument. Eight sampling points were selected in a portion of beach approximately 610m long. For each point, two pitfall traps were placed and remained active for 7 days, the first one placed at the surface level of the banquette and the second one at a depth of 50cm. In addition, two samples were taken for analysis by visual census and the other by Berlese-Tullgren funnel extraction. The sampling sessions were carried out approximately every 40 days from January 2024 to November 2024. Sampled individuals were identified at Order level. For spiders, a more detailed identification was conducted at Species level. GLMM models were developed to analyse abundances, the number of taxonomic groups and Simpson's indices, in function of seasons and sampling method, and a CCA analysis to highlight the relationships between taxonomic groups, seasons and sampling methods. The most significant seasonal variations are in the abundances of individuals captured, with the subterranean pitfall and Berlese-Tullgren funnel recording the largest numbers of individuals. For taxonomic groups and Simpson's indices, the differences are less marked or non-significant. Fifteen species of spiders were identified, including three new for Italy (*Dysdera cf. gamarrae* Ferrández, 1984; *Gnaphosa jucunda* Thorell, 1875; *Erigone dentosa* O. Pickard-Cambridge, 1894) and one new species for Latium (*Chaerea maritimus* Simon, 1884). The results obtained extend a still limited literature, helping to highlight the biological importance of Posidonia oceanica banquettes, which often remain underestimated and poorly studied.

Circadian and sleep-like rhythmicity in the scalloped hammerhead shark (*Sphyrna lewini*)

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Understanding biological rhythms in continuously swimming marine animals is crucial to elucidate their rest-activity balance and internal timekeeping. Scalloped hammerhead sharks (*Sphyrna lewini*) are ram ventilators with high energetic demands, and have recently been shown to display a rhythmic rolling swimming behaviour (RSB). We investigated the presence and function of biological rhythmicity in wild *S. lewini* using high-resolution accelerometry and time-series analysis. Our results reveal robust circadian rhythmicity in activity levels, with a consistent nocturnal acrophase and significant anticipation of light transitions, indicating entrainment by an internal circadian clock. Concurrently, we identified a structured ultradian rhythmicity in rolling behaviour, which differed markedly between day and night. Daytime rolling cycles were shorter, less frequent, and associated with low metabolic effort, suggesting a restorative function. Nighttime rolling was longer and more active, potentially supporting navigation and environmental sensing. We observed a homeostatic relationship between night and day rolling intensity, consistent with compensatory rest regulation. Additionally, we detected a modulatory effect of moonlight on nighttime activity and subsequent daytime rest, suggesting masking effects. Altogether, our findings support the presence of circadian and sleep-like processes in *S. lewini*, and raise new questions about how these rhythms support energy conservation, navigation, and sensory integration in pelagic predators.

Wild bees (Hymenoptera, Apoidea, Anthophila) and flowers: evaluation of visit frequencies in urban nature reserves

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Urbanisation is a pervasive global process that contributes to habitat fragmentation and biodiversity loss. Nevertheless, large urban green spaces can serve as important refuges for wildlife, offering critical habitats that support biodiversity conservation even within metropolitan contexts. Also in the urban ecosystem, wild bees represent key pollinators among urban fauna, playing a crucial role in ecosystem functioning. Their diversity and activity are closely linked to the availability and characteristics of flowering plants, which provide essential food resources. This study investigates the interactions between wild bees and spontaneous flowering plants within selected large urban nature reserves in Rome, Italy. Specifically, we analysed bee visitation frequencies at the genus and family level and examined the role of functional traits of flowers and bees in shaping these interactions. For bees, we considered traits such as the ligula's relative length and position of the scopa on the body. We focused on inflorescence type, corolla structure, and flower colour combinations for plants. Our goal was to characterise the structure of pollination networks in large urban green areas and to understand how morphological and functional traits of both bees and flowers influence interaction patterns. Preliminary results suggest that specific trait combinations are associated with higher visitation rates, indicating a degree of trait matching that may enhance pollination efficiency and network stability. Emerged that a few plant families are highly represented and visited by many bee species, while many other botanical groups are visited by small groups of wild bees. Regarding morpho-functional traits, long-ligula bees visit a wider range of flowers and preferentially visit deep and tubular flowers, while short-ligula bees visit a smaller number of plant taxa. From the analysis of scopa position to corolla structure, significant results didn't emerge. The findings highlight the importance of functional traits in mediating ecological interactions in urban ecosystems. This approach provides a deeper understanding of how urban green spaces can sustain complex pollination networks, offering practical insights for urban biodiversity management. By identifying the traits that facilitate effective plant–pollinator interactions, this research can inform targeted conservation strategies aimed at promoting pollinator diversity and abundance in cities.

Accumulation of trace elements and assessment of oxidative stress in three European eel (*Anguilla anguilla*) populations in Calabria

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The European eel (*Anguilla anguilla*), a widely distributed catadromous species currently classified as *Critically Endangered* by the IUCN, is experiencing a marked decline on a continental scale. This decline is attributable to multiple factors, including chemical contamination of aquatic ecosystems.

This study investigated the concentrations of 16 trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr, Zn, Ba, Bi) in the muscle tissue of 20 specimens collected from three sites in Calabria (Crati River, Raganello Stream, La Vota Lakes), using inductively coupled plasma mass spectrometry (ICP-MS).

Statistical analysis revealed significant differences in the bioaccumulation of 10 out of the 16 elements among the populations, raising particular concern over the significant results for cadmium and arsenic.

In parallel, ecotoxicological assays were conducted to assess oxidative stress (SOD, TBARS, ketonic and aldehydic OMPs) in four target tissues (muscle, heart, liver, gills). In muscle tissue, populations showed statistically significant differences for all biomarkers: OMP values were highest in La Vota Lakes, while SOD was highest in the Crati River.

Correlation analysis between bioaccumulation and stress revealed site-specific responses: no significant relationships were observed in Raganello; in the Crati, Mo and Cr were positively correlated with TBARS, while Zn was negatively correlated with SOD; in La Vota Lakes, Al was negatively correlated with TBARS, Bi was positively correlated with aldehydic OMPs, Se and Co were positively correlated with SOD, and Cr was negatively correlated with SOD.

The results highlight complex interactions between chemical contamination and physiological responses, underscoring the vulnerability of *A. anguilla* in ecotoxicologically critical environments.

“That’s a trap!”: Identifying extracellular traps formation in invertebrates and vertebrates

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All living animals possess multiple conserved mechanisms for regulated cell death (RCD) like apoptosis, but among these, the formation of extracellular traps (ETs) or ETosis is an innate immune mechanism to cope with the invasion of pathogens, recent studies have also shown how ETosis takes part into various auto-immune diseases. Although described in many vertebrates, few are the descriptions in crustacean, and almost none for echinoderms and sharks. Thus, this research characterized ETosis in four species: two invertebrates (*Cherax quadricarinatus*, Crustacea and *Arbacia lixula*, Echinodermata), as well as two vertebrates (*Cephaloscyllium umbratile* and *Triakis scyllium*, Chondrichthyes). Haemolymph, coelom or blood was extracted from *C. quadricarinatus*, *A. lixula*, *C. umbratile* and *T. scyllium*, then the respective immune cells were fractionated. Consecutively, to induce the release of ETs the cells were exposed in-vitro to Gram+ and Gram- bacteria: Escherichia coli or its LPS alone, S. aureus or L. garvieae (DNase or cytochalasin-D as positive controls). Lastly, fluorescent microscopy analyses and further quantification using ImageJ were performed. The results show species-specific differences in the morphology of the ETs and the time of release after exposure to each pathogen. Specifically, *C. umbratile* and *A. lixula* exhibited more diffuse ETs. Whilst in *T. scyllium* and *C. quadricarinatu*s, produced conspicuous ET filaments when induced by Gram- and Gram+ bacteria respectively. However, the amount of exDNA vary among species depending on the concentration and activator specifically, sharks undergo ETosis releasing a lower amount compared with invertebrates. Moreover, after analysing all the results obtained, mainly two ETs morphologies are the most common: filamentous and diffuse. Those results, according with previous studies, suggest an effective recognition and a extraneous molecules and consecutive clearance of the bacteria. Thus, this is the first comparative report among different taxa, assessing the highly specificity of ETosis in each organism, and how it is strictly influenced by the concentration of the stimulant. Overall, ETosis in both invertebrate and vertebrate’s immune cells was identified, suggesting it is a conserved RCD mechanism with insights for further studies on the specific activation and the implications on how this process can be regulated.

A spatio-temporally explicit distribution model to support conservation strategies for isolated small-ranged species: a case study from Galápagos

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Species Distribution Models (SDMs) represent the gold standard to investigate how environmental factors affect the occurrence of animal species and estimate their actual and potential distribution. However, SDMs often lack a formal hypothesis-driven selection of environmental predictors and require independence among observations. In contrast, actual occurrence data are often spatially and temporally autocorrelated, particularly when most observations originate from a limited number of surveys, as frequently happens in studies focusing on isolated, small-ranged species of high-conservation priority. Here, we explored the application of a hierarchical Bayesian spatio-temporal SDM to model the probability of occurrence of small-ranged species living in isolated and difficult-to-access areas, combining Integrated Nested Laplace Approximation (INLA) with Stochastic Partial Differential Equation (SPDE). To do so we used the Galápagos Pink Land Iguana (*Conolophus marthae*) as a model organism. This species is well suited for our scope in being critically endangered and endemic to an extremely small and remote area on Wolf Volcano on Isabela Island, Galápagos. Our approach enabled us to obtain reliable estimates of the effects of environmental variables on species distribution using opportunistic occurrence data, which exhibit a pronounced spatio-temporal structure. A spatially explicit cross-validation of the model demonstrated that the INLA-SPDE approach allows elucidating the relationship between small-ranged species and their areas of distribution, resulting in accurate predictions (Cohen's K = 0.86; True skill statistics = 0.84). We also used the INLA-SPDE model to map the actual and potential distribution of the species across the Galápagos archipelago. Our approach provided a first formal measure of the range of *C. marthae* (*ca.* 42 km2) and indicated that abundance of trophic resources, availability of open areas, and terrain roughness limit species' distribution. We also identified *ca.* 400 km2 of suitable areas clustered into three main sites outside the species' actual range. These results constitute an essential starting point to evaluate a species translocation, a key conservation strategy outlined in the Conservation and Management Plan for this species.

Phoresy by ants: despersion of Tardigrades enhanced by anhydrobiotic capabilities

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Phoresy is a temporary symbiotic relationship where an organism, generally with limited active dispersal capabilities, uses a more mobile host for dispersion to reach new habitats or resources. Tardigrades are known to use as phoretic hosts various vertebrates and invertebrates with limited mobility. This study investigated the potential role of ants as dispersal hosts for tardigrades, a possibility not yet explored, as similar behaviours have already been documented in ants with other invertebrates.

To verify the tardigrade-ant associations, a field survey was conducted on arboreal ants. Oak galls of the cynipid wasp *Andricus quercustozae* colonized by four ant species were collected, the presence of tardigrade in these ants' gall nests and on ant bodies were assessed. The transport of tardigrades by ants was also evaluated by isolating galls for one year and then exposing them to ant colonization, as well as by laboratory experiments on tardigrade phoresy focusing on the behaviour of the ant *Colobopsis truncata*.

Ants carried tardigrades on their bodies, providing the first evidence of ant-mediated tardigrade phoresy, especially with *C. truncata*. Moreover, ants transport moss and lichen fragments inside their nests, thus tardigrades and other meiofaunal organisms were retrieved in galls. The high humidity conditions within galls spongy material allow tardigrades to be alive and active. The intense foraging activity and long distance walked by ants together with the high number of potential "transporters" within colonies explain the diversity of the tardigrade species retrieved within the galls. Finally, desiccated tardigrades may withstand potential long-distance transport by ants. Indeed, animals able of undergoing anhydrobiosis could properly desiccate on the body of an ant or within fragments of substrates (e.g. mosses, lichens) and being transported for long distances withstanding extreme conditions.

Thanks to the ant widespread abundance, myrmecophoretic dispersal may contribute to the spread of meiofaunal organisms in terrestrial habitats. These findings represent just the "tip of the iceberg" of an unexplored passive dispersal modality over significant distances for terrestrial hydrophilic micrometazoans, broadening our understanding of ecological interactions and phoresy.

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Diversity and distribution of Apoidea across conventional and organic crops in the MAB-SILA UNESCO biosphere reserve (Calabria, Italy)

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Pollination sustains plants and is therefore pivotal for the environment. Wild bees (Hymenoptera, Apoidea, Anthophila) represent the most significant group of plant pollinators, with a total of over 20,000 species identified worldwide. Nonetheless, these species are in decline due to a range of stressors, including habitat fragmentation, pesticides and pollution. These factors primarily affect their diversity and abundance. In order to design effective conservation programmes, it is necessary to collect information about bee presence and distribution, however more than 50% of wild bee species in the IUCN Red List for Europe are data deficient. This underscores the need for more ecological studies on species distribution and diversity. Mediterranean countries have the most diverse bees, including more than 1,000 species in Italy. Nevertheless, data on wild bees in certain areas, such as Calabria, are either limited or lacking.

The present study was performed in a MAB area in Calabria (Italy), as part of the BIONETPARKS Project – Protecting Pollinators and Plant Species in the MAB-SILA UNESCO Biosphere Reserve. A total of four sampling sites were chosen in cultivated areas, of which two were under conventional management and two under organic management. The sampling method employed, in accordance with the EU Pollinator Monitoring Scheme (PoMS), was permanent transect walks. The monitoring period (2022-23) resulted in the identification of 38 species, which belonged to 14 genera and 4 families. The most prevalent genera were *Andrena*, with a total of 14 species documented, and *Lasioglossum*, which accounted for 7 species.

A diversity analysis was conducted, with the alpha and beta diversity indices employed as the primary analytical tools. Subsequently, these indices were correlated with agroforestry activity, temperature and altitude. Furthermore, the relative abundances of the various genera at different sites were taken into account, showing a greater abundance of social managed species (*Apis*, *Bombus*) in the conventional areas, while organic farms exhibited a greater abundance of wild bees (*Andrena*, *Lasioglossum*). In addition, the interaction between the identified wild bees and the host plants was analysed and an overview of the knowledge on the ecological characteristics and conservation status of the identified species was considered. This study presents a preliminary investigation into the current status and diversity of Apoidea in Calabria.

A national reference for wild bee identification: Italian adaptation of Michez et al. (2024) key to bee genera

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In recent years the interest and concern for nature and its safeguard has grown. The European Commission has adopted the Law for the Restoration of Nature, which establishes and guides member states in implementing measures to promote the conservation of biodiversity. Pollinators, and in particular wild bees, play a significant role in this plan because of their key role in the ecosystem functioning and the critical decline they are facing. Several European projects for monitoring and conservation of wild bees have been launched. These have encountered problems due to a lack of resources for taxonomic identification. It is therefore evident that there is a need for both information and tools for taxonomic identification, as well as for training a new generation of experts. Projects such ORBIT and SPRING were launched for this purpose. ORBIT was created to provide a tool to support the taxonomic identification of European wild bees, while SPRING project aimed to test the implementation of the EU Pollinator Monitoring Scheme (EU PoMS) for wild bees and other pollinators. In this framework an international team of experts is working on adapting at national scale the "Key to the genera of European bees (Hymenoptera: Anthophila)" (Michez et al., 2024), a reference identification tool, at genus level, for the Apoidea fauna of each European country. The purpose of this work is to introduce the Italian version of the key.

The Italian key, to be published as a volume, is an adaptation of the European key by Michez et al. (2024). It has been rearranged to focus only on the Italian fauna of Apoidea Anthophila, removing the genera not present in the peninsula. In Italy, we have 61 genera of the 77 European genera listed on the original key.

The volume encompasses a general overview of the life cycle, ecology and morphology of bees. It also summarises the families, subfamilies, tribes, and genera present in Italy, including the number of species and the main bibliographic references available for species-level identification. Finally, the work provides two illustrated identification keys, one for females and one for males, and also includes species factsheets covering some of the most common and representative Italian species. This work, intended for students but also enthusiasts or amateurs, aims to help facilitate the dissemination of taxonomic knowledge on Apoidea, and therefore, approach the monitoring and conservation goals set by the European Union.

Trophic shift in *Mustelus mustelus* and M*ustelus punctulatus*: a vertebral stable isotope approach

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The increasing vulnerability of demersal elasmobranchs to overfishing, and the consequent global decline of their populations and their ecological role as apex predators in epi-benthic ecosystems, has led to growing scientific interest in understanding their trophic ecology. This aspect is considered essential for developing effective conservation and management strategies, especially in areas subject to high anthropogenic pressure.

Stable isotope analysis has proven to be a powerful tool in this kind of investigation. Soft tissues such as muscle reflect dietary input over relatively short periods, weeks to a few months, due to their higher metabolic turnover. In contrast, hard tissues, like vertebrae, are known to incorporate isotopic signals more slowly, reflecting feeding patterns over months or even years. This slower turnover rate allows vertebral isotopes to serve as a biological archive, providing long-term insights into individual trophic histories and ecological changes across life stages.

In this study, we analysed the stable isotopes of nitrogen (^{1}N) and carbon (^{13}C) along the vertebrae of *Mustelus mustelus* and *Mustelus punctulatus*, two demersal shark species that are increasingly threatened in various Mediterranean regions. A total of thirty vertebrae from *M. mustelus* and thirty-five from *M. punctulatus* were examined using isotope ratio mass spectrometry, enabling the reconstruction of individual trophic trajectories across ontogeny. The results revealed distinct species-specific patterns. *M. punctulatus* exhibited a general increase in ^{1}N values with increasing body size, suggesting a trophic level shift as individuals grow, reflecting a dietary transition toward larger or higher-level prey. In contrast, *M. mustelus* showed stable isotopic signatures throughout ontogeny, indicating a more consistent diet over time and potentially a narrower ecological niche.

These findings highlight the value of vertebral stable isotope analysis in detecting ontogenetic trophic changes and contribute to a better understanding of the feeding ecology of demersal sharks. Such insights are fundamental to inform conservation policies and improve the management of species like *M. mustelus* and *M. punctulatus*, which are especially vulnerable in heavily exploited marine environments.

An habitat fight: the interaction between the polychaete *Sabellaria spinulosa* and the phanerogam *Posidonia oceanica*

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Posidonia oceanica (L.) Delile (1813) is the most important Mediterranean seagrass, and its meadows perform a pivotal role within the coastal marine environment. The polychaete *Sabellaria spinulosa* (Leuckart, 1849) is a biogenic reef builder in sandy environments and is included in the Red List of Marine Habitats and it is a priority habitat in the OSPAR convention. In the framework of the projects MOSSHA and NBFC, we analyze the interaction between the two habitat formers in an area adjacent to the Nature Reserve “Le Cesine”, in the Southern Adriatic Sea. The morpho-structural characteristics of *P. oceanica* and both the qualitative and the morphometric characteristics of *S. spinulosa* bioconstructions were analyzed in eight subareas where the two habitats co-occur and where they are isolated from each other. A visual and photographic census was conducted in each sub-area using a standard 40 x 40 cm reference square. *P. oceanica* was analyzed following ISPRA’s reference methodologies, while the analysis of the bioconstructions of *S. spinulosa* was performed by both non-destructive (video and photographic records) and destructive (collection of 18 blocks of 20 x 20 cm) sampling. The morpho-structural characteristics and the macrofauna associated with each block were determined. Density, coverage, and leaf production of *P. oceanica* exhibited higher values in absence of *Sabellaria*, showing instead a decline in the areas where interaction took place. The analysis of phenology and lepidochronology demonstrated a marked decline in the health status of the meadow in the presence of *Sabellaria*. Conversely, the qualitative analysis of S. spinulosa revealed that the presence of *P. oceanica* caused erosion, reduced compaction, more fractures, and reduced cover of epibionts. No effect of the interaction on the size of *S. spinulosa* individual was detected while their density was higher in the interaction areas. Finally, the macrofauna associated with the blocks was more abundant and diversified in the interaction areas. Thus, S. spinulosa tended to impair the growth of *Posidonia*, reducing the production and development of new rhizomes and causing a considerable decline in the productivity of the plant, while the interaction caused both positive and negative effects on the polychaete bioconstruction.

Bridging a two-decade gap: new insights into wild bee diversity of Circeo National Park (Latium, Italy)

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Circeo National Park (CNP) is one of the most important natural areas in Central Italy, encompassing a remarkable variety of habitats — including coastal dunes, wetlands, forests, and agricultural landscapes — and preserving numerous endemisms. Among its faunal communities, wild bees represent key pollinators, providing essential ecosystem services that sustain both plant populations and agricultural production. Despite their ecological importance, their diversity within the CNP has been scarcely documented over time. In fact, the last systematic surveys date back to two distinct periods: the first in 1963, and a later campaign between 1993 and 1998. Since then, no comprehensive monitoring has been conducted. The present study is part of the national framework of actions titled "*Pollinating Insects: Biodiversity and Ecosystem Services*", promoted and funded by the Italian Ministry of the Environment (MASE) through the 2019 Directive addressed to Italy's National Parks and Marine Protected Areas, and aims to investigate current wild bee diversity across different environments of the CNP. Field sampling was carried out from March to September over three consecutive years (2021–2023). Bees were collected using entomological nets along fixed transects (200x4m), evenly distributed across three main habitat types: agricultural areas with varying management practices, coastal sand dunes, and the Circeo Promontory. The data obtained are compared with results from previous surveys to assess changes in species composition. Moreover, differences in wild bee communities among the sampled habitats are discussed. This study contributes to updating the knowledge on the current status of wild bees within CNP, providing essential data to support conservation strategies.

Peracarida fouling communities along a Mediterranean North-South axis

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Marine ecosystems, including marinas and harbours, are hotspots for Non-Indigenous Species (NIS), which pose significant threats to marine biodiversity. Boating activities are a major vector for the introduction of NIS, particularly Peracarida, whose natural dispersal capabilities are limited. This work aimed to examine the variability and dynamism of Peracarida fouling fauna in three Italian key sites: Palermo and Trapani marinas, both highly impacted by recreational boating and located near the Ustica and Egadi marine protected areas (MPA), and the marina of Licata. Two additional sites were sampled for the first time: the Bergeggi MPA, Italy, and the Kerkennah archipelago in Tunisia, enabling a comparative analysis of communities along a north-south latitudinal and anthropogenic impact gradient. In addition to simply assessing species diversity, the study also considered the species' biological traits. A total of 25 species were identified, including eight NIS or cryptogenic species. The NIS *Caprella scaura* Templeton, 1836 was abundant in all marinas and surpassing in both abundance and frequency the other present predators, *Phtisica marina* Slabber, 1769 and NIS *Paranthura japonica* Richardson, 1909, likely due to its plasticity of feeding behaviour, being able to rely on filter-feeding when fully matured instead of being limited to carnivorous predation. The cryptogenic grazer *Jassa slatteryi* Conlan, 1990, efficient in detritus generation, was found only in Trapani. Wherever *J. slatteryi* was absent, we found the detritivorous *Eritchtonius brasiliensis* (Dana, 1853) and NIS *Stenothoe georgiana* Bynum & Fox, 1977, in Palermo, Licata and a sampled pier in Trapani. The highly invasive detritivorous NIS *Laticorophium baconi* (Shoemaker, 1934) was found at all sites except one in Licata, where the NIS *Monocorophium acherusicum* (A. Costa, 1853) occured. NIS *Paracerceis sculpta* (Holmes, 1904) was absent from all Trapani piers. In Kerkennah, NIS *Ampithoe bizseli* Özaydinli & Coleman, 2012 was recorded; in Bergeggi *S. georgiana* was present. Fouling community structures and NIS assemblage varied not only among marinas but also between individual piers in Trapani and Licata. These differences were not linked to seasonality. Across all sites, species showed a broad range of trophic strategies, with suspension-feeders dominating the community structure.

Experimental setup for the study of behavioral responses of *Octopus vulgaris* to acoustic stimuli

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The sea is a complex acoustic environment, encompassing a wide range of sounds - from natural to anthropogenic sources - that influence how marine organisms communicate, navigate, and detect predators or prey. Although marine vertebrates have been extensively studied in this context, the role of sound in the sensory perception of cephalopods, and particularly in *Octopus vulgaris*, still remains poorly understood. Physiological evidence indicates a potential sensitivity to low-frequency sounds (< 400 Hz), but systematic behavioral studies are lacking.

The aim of this work is to develop an experimental protocol to evaluate the behavioral responses of *O. vulgaris* to artificial and natural sound stimuli in an acoustically controlled environment. We designed a dedicated experimental tank to perform a comprehensive assessment of animal behavior under different sound stimuli across a wide range of frequencies and sound pressure levels, monitoring background noise levels. The behavioral responses of individual *O. vulgaris* to each sound combination were categorized as follows: no response, posture, startle, body pattern change, arm movements, grooming, jetting, and inking. These responses are plotted against stimulus conditions for the construction of a behavioral audiogram.

This experimental approach allows us to explore the octopus's auditory capabilities and contribute to a better understanding of its sensory strategies, with potential implications in field ethology, zoology and conservation.

Tracking chromosomal damage across species: the micronucleus assay as a versatile biomarker

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The micronucleus (MN) assay is a cytogenetic technique that allows to detect small, extra-nuclear bodies (‘micronuclei’), arising from an incorrect integration of chromosomes or chromosome fragments into daughter nuclei during cell division. The technique is widely applied to evaluate chromosomal damage resulting from exposure to environmental pollutants or physical stress. Our primary aim is to review how the assay has been applied across diverse aquatic and terrestrial animals. Moreover, here we develop two new protocols to further broaden the applicability of the test, on the freshwater snail *Lymnaea stagnalis* and on the invasive blue crab *Callinectes sapidus*.

Methodologically, the MN assay involves collecting specimens—whether from controlled laboratory cultures or from the field—from which cells are gathered, fixed, and stained on lab slides. Typically examined tissues are buccal mucosa and circulatory tissues like blood (for vertebrates) and hemolymph (for invertebrates). Microscopic examination of the lab slides then allows to score the frequency of micronuclei or other nuclear aberrations. Focusing on size, shape, and staining intensity, these structures can be discerned from the main nucleus of from staining artifacts.

This method’s simplicity and cost-effectiveness have allowed it to be successfully employed on many vertebrates and invertebrates, revealing a wide spectrum of genotoxic responses, such as those caused by some industrial waste, pesticides, and heavy metals. Fish and amphibians generally represent sensitive models due to their permeable tissues and are tested through straightforward blood sampling, while terrestrial mammals have often been sampled using mucosal cells. Even invertebrates have provided valuable data, despite some sampling challenges. *L. stagnalis*’ behaviour of extruding hemolymph when prodding the animal’s foot with a micropipette makes it particularly suitable for this test, while *C. sapidus*’ invasive nature in the Mediterranean makes it a widespread and easily accessible resource as well.

Overall, the assay is a valuable tool for environmental monitoring and risk assessment through the use of various taxa, and findings from *L. stagnalis* and *C. sapidus* further confirm its versatility. By implementing robust methodologies and exploring its application to new organisms, it is possible to exploit the assay to detect the effects of genotoxic pollutants in various environments.

Macrozoobenthic non-indigenous species colonization in a Mediterranean Coastal Lagoon: comparison between natural and anthropogenic substrates

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Macrozoobenthic coastal communities are among the most productive in marine ecosystems, often threated by biological invasions. Proliferation of non-indigenous species (NIS) outside their natural boundaries depends on several factors, including life-cycle, physiological plasticity, introduction pathways and substrates availability. The aim of this study is to compare the variability of hard bottom macrozoobenthic diversity and NIS abundance related to substrate type. Sampling was carried out in the Natural Oriented Reserve of Capo Peloro Lagoon (Messina, Italy) in 2023, collecting 115 marine litter objects (Anthropogenic Substrate-AS) and 115 *Pinctada radiata* (Natural Substrate-NS). NSs and ASs were transported in laboratory and sorted to collect the associated macrozoobenthic fauna. A total of 201 associated species were identified in both the substrate types, belonging to Polychaeta (85 species), Crustacea (49), Mollusca (41), Echinodermata (10) and 16 species belonging to minor represented groups. Nineteen NIS were identified during the analysis, representing almost the 10% of the total number of detected species. Concerning abundances, NIS represented the 4.5% of the total macrozoobenthos abundance, with the 95% of NIS abundance detected from NS. SIMPER analysis based on substrate typology showed an average similarity of 16.07% for AS, due to high abundance of *Pileolaria pseudomilitaris*, *Aiptasia mutabilis* and *Janua heterostropha*, and 33.91% for NS, given by Serpulidae and *A. mutabilis* as well; dissimilarity between substrates was of 90.98%, with NIS contributing for the 11.73%. *Hydroides dianthus*, *Paracerceis sculpta* and *Dorvillea similis* were the most responsible for the detected dissimilarity. Diversity was explored through a species abundance Bray-Curtis similarity matrix followed by Hierarchical Cluster analysis and Multidimensional Scaling. Results showed the 40% of similarity among natural and anthropogenic substrates, and the 60% among the same substrates from different sampling sites. Findings from the present research confirmed the influence of substrates type and sampling site on macrozoobenthic assemblage composition and abundance. Considering the sensitivity of coastal lagoons to anthropogenic pressure, monitor the NIS spreading inside these ecosystems, and improve the knowledge base on their distribution and abundance, is essential to plan effective management actions to face and prevent potential negative impacts of biological invasion.

Otolith variability, age composition, and ontogenetic diet shift in *Trachurus trachurus* , Linnaeus, 1758, from the Southern Tyrrhenian Sea

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Overexploitation of marine resources poses a global threat to biodiversity and food security, with the Mediterranean Sea particularly affected due to intense fishing activity and multiple anthropogenic pressures. This study provides new insights into ontogenetic changes in diet and otolith morphology of *Trachurus trachurus*, a commercially important Mediterranean species. A total of 282 specimens were collected from the commercial fishery in the Gulf of Palermo (Southern Tyrrhenian Sea) for analyses of otolith morphometry, age structure, and diet composition. Otoliths were analysed using wavelet transformation methods, and age was determined by counting annual growth zones, each consisting of one translucent and one opaque ring. The diet composition was assessed through stomach content analysis. Wavelet analysis revealed clear differences in rostrum length, notch depth, and posterior dorsal margin, with six distinct morphotypes identified in the sample. Age composition was dominated by juveniles and intermediate-aged fish: 38.7% belonged to age group 1, 46.8% to age 2, 13% to age 3, and only 1.5% to ages 4–6. Dietary analysis revealed a total of 1,418 prey items from 33 taxa, with 254 stomachs containing food remains and 28 being empty. It was also examined the diet variability in relation to specimens age, revealing an ontogenetic dietary shift: : juveniles (ages 1-1+) primarily consumed small pelagic crustaceans, individuals aged 2- 2+ included more fish and decapods, and older fish (ages 3-6) specialised in larger crustaceans and fishes. These results demonstrate dietary specialization and trophic flexibility across age classes in response to resources availability. These findings highlight the importance of integrating morphological, age, and dietary data to better understand life history strategies and ecological dynamics in commercially exploited fish species.

Early-stage Medaka Embrioys (*Oryzias latipe*s) as an alternative model for evaluating the toxicity of of bromuconazole in vivo

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Bromuconazole (BRO) is a triazole class of chemical fungicides widely used worldwide since the 1970s. BRO is currently authorized as a Plant Protection Product (PPP) under Regulation EC 1107/2009, with approval renewed until 30/04/2027 in 14 EU Member States. Despite its ongoing utilisation, the substance has been designated as an EU candidate for substitution, as it fulfils the criteria of "Persistent-Bioaccumulative-Toxic" according to the EU Pesticide Database and EFSA evaluations, namely with respect to its persistence in the environment and its toxicity to aquatic organisms. Due to concerns about its ecotoxicity, BRO has been included in the 5th Watch List established under the EU Water Framework Directive (2000/60/EC), which identifies emerging pollutants that require further environmental monitoring. BRO's Predicted No-Effect Concentration (PNEC) for aquatic environments is 0.015 µg/L. Recent studies have shown that exposure to BRO may cause cardiotoxicity, oxidative stress, and lipid metabolism disorders in zebrafish (*Danio rerio*) larvae and adults. This finding raises further concerns about potential consequences of its use on aquatic organisms (Huang et al., 2024; Qin et al., 2022). Therefore, the current study aims to assess the effects of BRO on the development of medaka (*Oryzias latipes*) from early stage to 5 days post-fertilization (dpf). Medaka is particularly suitable for toxicity study due to the optical transparency of the eggs, rapid embryonic development, and the high sensitivity to environmental contaminants at early stages of its development. After determining the Lethal Concentration 50 (LC50) through acute toxicity tests, the embryos were exposed to various concentrations of BRO to assess potential effects on early development. The dose selection was based on environmentally relevant levels and available literature data. Morphological and functional endpoints such as survival, somite development, heartbeat, and overall embryonic morphology were evaluated. This approach represents a valuable tool for the early toxicological assessment of persistent fungicides such as Bromuconazole, providing the basis for future studies on their environmental impact and safety profiles. Furthermore, the use of medaka embryos up to 5 dpf allows for in vivo testing in compliance with the 3Rs principles and EU Directive 2010/63/EU.

Effects of environmental microplastics on soil microarthropods survival, behaviour biodiversity

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Microplastics (MP, min di 1 mm) are ubiquitous in terrestrial ecosystems. Their presence is associated with potential alterations in the physico-chemical characteristics of soil and with impacts on the behavior and survival of soil organisms. Nevertheless, to date, studies on the effects of MP have mostly been limited to model organisms, such as the collembolan *Folsomia candida.*

In this study, the effects of MP derived from the degradation of plastics present in urban environments were assessed on different species of soil microarthropods. MP were tested on three wild collembolan species (*Parisotoma notabilis*, *Onychiurus* sp., and *Ceratophysella denticulata*), on the model species *F. candida*, and on the oribatid mite *Scheloribates* sp. The wild species used were acclimated in the laboratory for 2 years before testing, achieving stable populations.

The MP tested originated from HDPE paving blocks commonly used in urban parking areas, which were fragmented in the laboratory to obtain the sub-millimetric fraction (10–832 µm) used in the tests. The effects of MP (at 1000 mg/kg) on microarthropod behavior were evaluated with ISO avoidance tests, while the effects on survival were assessed in *P. notabilis* and *F. candida* using OECD chronic exposure tests, exposing organisms to increasing concentrations of MP (from 10 mg/kg up to 1000 mg/kg). The standardized protocol for *F. candida* (28 days and 20 °C) was modified for the wild species *P. notabilis* based on its life cycle characteristics, extending the duration of the test to 35 days and setting the temperature at 17 °C.

The results show a clear trend of avoidance of MP-contaminated soil by all tested microarthropod species, with significant differences compared to the control groups. *F. candida* proved to be the least sensitive species to the presence of MP (avoidance of 34 ± 13%), while the highest values were recorded for the collembolan *Onychiurus* sp. and the mite *Scheloribates* sp., with avoidance rates of 53 ± 13% and 53 ± 19%, respectively.

In chronic exposure experiments (28–35 days), a significant decrease in collembolan survival was observed in response to MP exposure, with mortality rates at the highest concentration tested reaching 57.8 ± 13% in *F. candida* and 92.2 ± 4.4% in *P. notabilis.*

This study contributes to understanding and quantifying the effects that MP present in terrestrial environments have on both model microarthropod species and wild species endemic to the Italian territory.

Conservation insights from morphometric and demographic monitoring of *Patella ferruginea* in Northen Sardinan MAPS

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Patella ferruginea Gmelin, 1791 is an endemic limpet of the Mediterranean Sea and is currently considered the most endangered marine species. Its distribution has been drastically reduced by human collection for ornamental, food and bait purposes and is now largely restricted to protected or inaccessible coastal areas. In this context, long-term monitoring is essential to support effective conservation strategies. This study, to be conducted between 2024 and 2026, aims to assess the population density and structure of *P. ferruginea* in La Maddalena Archipelago National Park through a complete census of the smaller islands and representative transects on the main islands. All individuals were and measured using biometric parameters (length, width, height). Morphotypes (*rouxi* vs. *lamarcki*) were identified based on the ratio of shell height to shell length, and sex was determined based on the length of the shell.

During the first survey phase (summer–autumn 2024), a coastal section of 3,709 meters was surveyed, in which 205 individuals were recorded, corresponding to a linear density of approximately 0.055 individuals per meter. The *rouxi* morphotype was dominant with 175 individuals (85%). Statistical analyzes revealed significant differences in size metrics and sex ratios between sites. The population in La Maddalena had the highest proportion of females (78%) with 160 individuals, 35 males and 10 immature individuals. The data were compared with other data from previous surveys (2018) in two other MPAs in northern Sardinia: Asinara NP and Tavolara MPA. All sites showed a similar dominance of morphotypes. However, there were significant differences in biometric variables and sex composition. Specifically, the mean shell length in Tavolara was 49.6 ± 17.1 mm, lower than in Asinara (53 ± 17.8 mm) and La Maddalena (50.7 ± 14.7 mm). Tavolara also had the highest proportion of males (27.4%), which probably affected the size differences, as males are generally smaller than females. The distribution of immature individuals was the same at all sites and did not appear to affect morphometric variability.

These results emphasise the importance of including morphometric and demographic data in conservation efforts. Continuous monitoring of *P. ferruginea* populations is crucial to identify local vulnerabilities and initiate management measures to conserve one of the most endangered endemic marine invertebrates of the Mediterranean.

From degradation to recovery: 30 years of illegal date mussel fishing impact on Apulian rocky shores

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This research evaluated the environmental consequences of the illegal harvesting of date mussels (*Lithophaga lithophaga*) along the Apulian coastline. The study primarily investigated the spatial distribution of habitat degradation, the formation of barren grounds, and the relationship with the population densities of sea urchins, specifically *Paracentrotus lividus* and *Arbacia lixula*. Between summer 2023 and winter 2024, a total of 42 locations were monitored, organized into six distinct sectors, covering an area of 59,196 m^2. The findings indicated a generally low impact of date mussel extraction, with a mean Damage Index (Dw) of 0.07, suggesting that recent disturbances are limited and localized. Nonetheless, sporadic instances of more recent damage were detected in both the Ionian and Adriatic Seas. The Rock Desertification Index (RDI) revealed the presence of barren grounds, particularly pronounced in the Ionian sector, where elevated sea urchin densities were recorded in the most severely affected areas. Statistical analysis demonstrated a strong association between sea urchin abundance and barren grounds, with larger individuals appearing to stabilize these degraded zones, while smaller specimens were linked to the freshly impacted areas from *L. lithophaga* exploitation. This study underlines the necessity of continuous monitoring and proactive conservation to safeguard biodiversity and the ecological balance of Mediterranean coastal environments. Additionally, it provides an updated cartographic representation of the current state of rocky coastal zones, forming a foundation for forthcoming ecological restoration initiatives.

Exploring neglected marine invertebrate fauna in Tunisian ports and marinas: NIS, rare taxa and first records

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The introduction and spread of Non-Indigenous Species (NIS) are widely recognized as a primary global threat to native biodiversity, with variable consequences by regions and ecosystems. Maritime transport and global warming are key drivers of this process, primarily through vectors associated with international shipping. The Mediterranean Sea, hosting over 6% of all known marine species, is both a biodiversity hotspot and a major NIS introduction zone, particularly in ports and marinas. Owing to its central position in the Mediterranean basin and relatively warm waters, Tunisia represents a strategic region for NIS monitoring and dispersal dynamics, notably related either to Lessepsian species advancing westward from the eastern Levant Sea, and to taxa introduced through the Gibraltar Strait. However, comprehensive studies of NIS in Tunisian waters remain scarce, particularly for small invertebrate taxa, whose status is still poorly documented. To address this gap, eight Tunisian ports and marinas (Marina Bizerte, Sidi Bou Said, Port Yasmine Hammamet, Port el Kantaoui, Sousse port, Marina Cap Monastir, Sidi Mansour, Sfax port) were surveyed in April 2025. At each site, a standardized sample of four liters of fouling organisms was collected from artificial substrates, including concrete walls, floating pontoons, mooring lines, and buoys, using surface-operated scraping tools. Samples were sorted, organisms photographed alive, preserved in 80% ethanol, and identified via integrative taxonomic method. This survey yielded new records of NIS and rare native species within Heterobranchia (Mollusca: Gastropoda), Peracarida (Arthropoda: Crustacea), and Polychaeta (Annelida), increasing our knowledge on the marine invertebrate fauna of Tunisia, particularly on the marine invertebrate diversity and distribution patterns in port environments, paving the way for future biogeographic and comparative studies. Furthermore, filling gaps on NIS distribution across the Mediterranean is critical for achieving effective biodiversity management and conservation planning.

Antennal sensilla equipment in the Apoidea: morphology, diversity and proposition of a consistent terminology

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Insect sensilla are the structural units responsible for the perception of chemical, hygro-thermal and mechanical stimuli, and they are especially abundant in the antennae. A number of studies have reported that the *Antennal sensilla* play a critical role in kin recognition, detection of natural enemies, foraging activity and mating activity. Given the wide range of ecological diversity of insects, it is not surprising that differences in antennal sensory system were reported between species with different life-history traits and within species between sexes. In the Apoidea (Hymenoptera: Aculeata), a large group including bees and stinging wasps, the morphology and distribution of *A. sensilla* have been investigated in several species, though the level of detail is very variable and quantitative analyses are rare. Moreover, entire lineages have not yet been investigated. We present here new data from a Scanning Electron Microscopy (SEM) analysis of *A. sensilla* in 14 species of bees, spanning six of the seven known bee families (Andrenidae, Halictidae, Colletidae, Apidae, Megachilidae and Melittidae), including lineages that have been particularly underrepresented in previous studies. A total of six types of *Sensilla* are present in the studied bee species: *Sensilla trichoidea* (ST, with three subtypes), *S. basiconica* (SB), *S. placodea* (SP), *S. coeloconica* (CO), *S. ampullacea* (AM) and *S. campaniformia* (CA). All these sensillar types occurred in the studied species. However, there was considerable variation in their density, which can be partly explained by body size variation. Indeed, head width (a proxy for body size) positively affected both antennal length and density of several sensillar types. Some differences among species, however, seem to be unrelated with variation in body size and may be linked to differences in life-history traits such as pollen specialization. These findings provide a foundation for more detailed investigations into the ecological pressures that may have influenced the evolution of antennal sensillar equipment in the Apoidea. Furthermore, this work has also given us the opportunity to review the currently available information on the antennal sensilla of Apoidea with the aim to address issues, such as the lack of a standardised terminology for sensillar types, which is essential for future comparative studies.

The Italian wall lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) as a bioindicator of environmental quality in three Calabrian sites with different degrees of anthropic disturbance

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Podarcis siculus (Rafinesque-Schmaltz, 1810) is a synanthropic species widely distributed and often used as an environmental bioindicator. This study evaluated its role as a bioindicator of the ecotoxicological quality of environments placed under different degrees of anthropization, verifying both the effectiveness of indirect methods such as leukocyte count, and of minimally invasive methods, such as the use of the tail, to analyze the different environmental conditions and quantify the levels of trace elements. Two sites were identified in the municipality of Rende (CS, Calabria): the Industrial Zone (IZ) and the Botanical Garden of the University of Calabria (BO); a third control site was located in the municipality of Fagnano Castello (CS, Calabria). From 2022 to 2024, five samplings were carried out per site on a seasonal basis, allowing a total of 250 lizards to be captured. Sex, age, morphometric measurements (SVL, TL, weight), ectoparasite load were recorded for each individual, leukocytes (basophilic, heterophilic, eosinophilic) and haemoparasites were counted. At the same time, pectoral muscles and unregenerated tails were analyzed for 30 individuals (10 per population) to evaluate their concentration of trace elements by ICP-MS spectrometry. In the ZI, individuals are larger and have a higher leukocyte load than those in BO; in both sites, females show higher leukocyte values than males. Lizards in BO have greater ectoparasite diversity and haemoparasite abundance than in the ZI; no significant difference between parasite load and leukocyte and emerged response. Regarding bioaccumulation, there were no statistically significant differences between males and females, except for copper. For the three populations, however, it emerged that for As, Mn, Fe, Cu, Mo and Ba the differences were statistically significant; elements such as As and Mo increased with increasing anthropization. Thus, the tail can actually be used as a substitute for muscle, but only for some elements of all those evaluated and cited previously, such as As, Se, Sr, Mo and Ba. *Podarcis siculus* confirms itself as an effective bioindicator for all three types of analyses carried out, capable of highlighting differences in the state of health of environments placed under different pressures. Caudal sampling represents a valid alternative to more invasive analyses.

Out of sight, out of mind? A Sicilian case study on the distribution of less charismatic species

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Addressing the biodiversity crisis requires an accurate knowledge of species distribution and their conservation status. However, our current knowledge remains largely fragmented, hampered by several well-known "biodiversity shortfalls", including the Linnean and Wallacean ones. These knowledge gaps particularly affect the so-called neglected species, i.e., those species that are less charismatic and often excluded from research and conservation priorities, despite their diversity and potential ecological roles.

Sicily is a recognised biodiversity hotspot in the Mediterranean area with a high number of endemic species. In this context, we investigated the inland water copepod fauna in a small area of northwestern Sicily, i.e., the San Vito Peninsula, with the explicit aim of assessing species richness in a restricted sampling area, estimating expected species occurrence, and evaluating the sampling effort required for an exhaustive faunal survey.

Through extensive sampling over two years, we identified 18 taxa, i.e., approximately 42% of the freshwater copepod species known for the entire island, also finding one species previously unrecorded in Sicily. These results, obtained in such a geographically restricted area (about 165 km^2), highlight the outstanding biological diversity currently overlooked, and often hidden within seemingly "minor" areas and habitats. Moreover, species accumulation curves, analysed using different asymptotic species richness estimators based on occurrence data, pointed out that the real copepod species richness of the area is expected to be considerably higher than what currently observed, suggesting that many species may have gone undetected despite the intensive sampling effort we realised.

These findings highlight the need of extending scientific surveys dealing with neglected taxa and environments. In fact, small water bodies, often overlooked in conservation agendas, actually represent biodiversity hotspots that harbour rich, unique and often vulnerable faunal communities. Therefore, it is essential to allocate sufficient resources to the systematic study of these habitats in order to close existing knowledge gaps and strengthen conservation strategies from an inclusive perspective on biological diversity.

Effects of heavy metal contamination on *Hermetia illucens* (Diptera: Stratiomyidae) physiology and development

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The larvae of the black soldier fly (BSFL), *Hermetia illucens* (Diptera: Stratiomyidae), are widely used to reduce organic waste and produce a protein-rich insect biomass which has potential applications in various industrial fields. However, contaminated substrates may expose BSFL to numerous pollutants, including persistent xenobiotics as heavy metals (HMs), which can not only negatively affect larval development and health, but also accumulate in insect tissues and organs, raising concerns about their potential transfer into BSF-derived products.

To explore the effects of HMs on insect growth, physiology, and immunity, BSFL were reared on the organic fraction of municipal solid waste supplemented with two PbCl concentrations (15 and 1000 mg/kg of diet), and their development and survival rates were monitored over the time. Additionally, markers of the cellular (hemocyte count and phagocytosis) and humoral (antimicrobial and lysozyme activities) branches of the immune system were analyzed. Finally, lead accumulation in whole larvae, midgut epithelium, and rearing residue (the so-called frass) was measured at the end of the bioconversion process.

The results revealed a concentration-dependent accumulation of lead in the larval tissue. The exposure to the highest PbCl concentration caused reduced weight and higher mortality rates, indicating the strong toxic effects of lead on BSFL. Moreover, the activation of both cellular and humoral immune responses was observed, highlighting negative impacts of heavy metal exposure on larval health.

This research offers valuable insights into how BSFL react to HMs contamination, reinforcing the evidence of lead's toxicity on this insect, and emphasizing the importance of thoroughly assessing the safety of using BSFL for the bioconversion of contaminated-substrate bioconversion for industrial applications.