EVOLUZIONE 2024

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# Book of Abstracts - SIBE2024

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# Opening Lecture

## Evolution and Ecology of Aging

Dario Riccardo Valenzano

*Leibniz Institute on Aging - Fritz Lipmann Institute, Jena, Germany*

Killifishes have emerged over the past few years as a powerful model system to answer open questions in biology of aging, developmental and evolutionary biology. Killifish evolved in a range of environments, from rainforest to savannah water holes that desiccate seasonally. Annual killifish survive periodic desiccation by evolving an annual life cycle, characterized by a specialized embryonic adaptation (embryonic diapause), where embryos suspend development in the dry mud until the external conditions are safe for them to hatch. Annual killifish that evolved in dry environments often display short natural lifespan and a wide range of age-related changes, including neurodegeneration, inflammation, fibrosis, immune decline and dysbiosis. In my talk, I will share how studying killifish ecology and evolution has opened new perspectives to understanding that species' lifespan and aging evolve as a function of past demographic constraints. We further generalize our findings using a numerical model and show that limited population size, combined with contingent ecological constraints (e.g. non overlapping generations and intermittent water availability) conditions phenotype evolution.

# Cavalli-Sforza Lecture

## Public health in the light of evolution

Tanja Stadler

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Pathogens evolve through time. This may result in changes in the rate of spread, either because mutations make the pathogen more transmissible or mutations escape immunity against infection. Further, evolutionary changes may alter the severity of infections. Pathogen sequences combined with laboratory and clinical data can shed light on such changes. I will give an overview of how we collected and analyzed SARS-CoV-2 sequencing data throughout the pandemic to provide information on the anticipated future fate of the pandemic. In particular, I explain how this data allowed us to accurately anticipate the Alpha and Omicron waves well before their occurrences. Further, we could quantify the increased severity of Delta. Such insights were -- in addition to sharing scientific preprints – communicated to the Swiss public health authorities and the government, allowing them to adapt their strategies. Most recently, with fewer resources going into sequencing individual-level patient samples, we co-founded a consortium to collect, characterize, sequence, and analyze a range of viruses on the population level in wastewater. I will discuss the scientific advances enabling us to shed light on the evolutionary dynamics of the different viruses based on wastewater. These advances are leveraged within a collaborative monitoring program where we join forces with public health authorities. Taken together, the presented data, tools, and results show that stable monitoring programs combining individual-level patient samples and population-level environmental samples will enable a more timely public health response to new pathogenic threats.

# Species adaptive response against climate changes

INVITED LECTURE

## Assisting seagrass forests to evolve from victims to allies against climate change

Lazaro Marin Guirao

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Seagrasses are higher plants that, after evolving on land, recolonized the marine environment forming extensive meadows in shallow waters of all continents. These meadows perform important ecological functions and provide numerous ecosystem services, making them one of the most valuable ecosystems on earth. Their huge capacity to sequester carbon and their role as a refuge for calcareous organisms, by buffering ocean acidification, make seagrasses a key ally in addressing the threats of climate change. However, seagrasses are currently victims of climate change and many populations have already shown signs of decline due to the increasing incidence of marine heat waves (MHWs). Meadows of the Mediterranean endemic species *Posidonia oceanica* represent the climax habitat along the coastline. The functional extinction of these meadows, as a consequence of warming, was predicted for the second half of this century, which has prompted scientific research over the last decade to try to reverse this fatal fate. Early studies on the heat stress response of *P. oceanica* from different thermal environments opened the door to the study of the (epi)genetic modifications behind the thermal tolerance of the species. In order to increase the resilience to MHWs, assisted-evolution techniques were further explored also on other species. Despite the encouraging results of pioneer works, recent studies have shown that plant "training" through exposure to "simulations" is not a trivial task and there is still a long road ahead before its effective application in the real world. Other assisted-evolution strategies such as chemical priming, selection of resistant genotypes/ecotypes or modification of the plant-associated microbiome are also currently being studied in seagrasses. The development of these techniques would increase restoration success of these crucial ecosystems, increasing their resilience as well as reinforcing natural populations. This new field of research offers an optimistic vision for the future of seagrasses in a rapidly changing world.

TALK

# Living beyond the edge: impacts of climate change on rock lizards at the niche margin

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Ectotherms are particularly threatened by climate change because they are strictly related to environmental conditions for their homeostatic mechanisms. In a scenario of global warming, ectotherm populations living in sites at the warm edge of the species' thermal niche may be pushed beyond this edge, suffering a disruption of thermoregulation processes, and detrimental effects at the population level. This study aims to test these predictions by collecting individual measurements and temperature data for the Bedriaga's rock lizard (*Archaeolacerta bedriagae)* across the entire species' range (Sardinia and Corsica). We compared lizard thermoregulation effectiveness (E), body condition index (BCI), and population size under different climatic conditions and during two sampling periods about 20 years apart. Results indicate that E, BCI, and population size vary across sites and time. E and BCI follow a linear pattern along the thermal niche gradient (Niche Margin Effect, NME) until a threshold temperature, beyond which the NME is disrupted. This threshold temperature represents the warm edge of the species' thermal niche. During a time frame of about 20 years three sites had moderate demographic variations, while two sites had a collapse of their population size. These two sites are those with the warmest climatic conditions. Interestingly, the climatic threshold separating the two groups of sites is slightly higher than those causing the disruption of the NME for thermoregulation effectiveness and BCI, suggesting that the individual inability to cope with extreme climatic conditions anticipate the demographic collapse. Our findings fully confirmed the hypothesis that a disruption of the homeostatic mechanisms takes place when the warm margin of the thermal niche is reached and is followed by a demographic decline. The approach implemented in our study is useful to identify the niche edge of a species and to predict which sites are prone to demographic collapses in the near future. This might allow to plan appropriate mitigation measures and management strategies to avoid local extinctions.

TALK

# Heavy metal pollution reduces resilience to climate change in mangrove crabs: a case study from Hong Kong

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Although the rate of mangrove destruction is slowing, the urbanisation and industrialisation of coastlines is causing unprecedented pollution in mangroves. Hong Kong's mangroves are no exception, receiving inorganic and organic input from domestic, agricultural, and industrial activities. Pollution by heavy metals is a serious concern due to their toxicity, persistence, and bioaccumulation. Heavy metal contamination is commonly assessed in mangrove sediments, but current research fails to consider their bioaccumulation and effects in local flora and fauna. Mangrove crabs are vital to the health and resilience of mangroves by performing key functional roles and metal toxicity experienced by these organisms may result in consequences for the wider ecosystem. By analysing the concentration of Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) we determined the spatial variation of heavy metal contamination and bioaccumulation in the sediments of five mangrove stands across Hong Kong. We then explored the physiological influence of heavy metals on keystone species by comparing the thermal tolerance of the resident crab species *Parasesarma continentale* exposed to varying levels of metal pollution in situ by analysing oxygen consumption and cardiac activity. The suppression of oxygen consumption and a reduced thermal optimum observed in *P. continentale* from the most polluted sites indicated significant narrowing and shifting thermal windows in crabs exposed to metal pollution, reducing their resilience to future climate change. Our findings highlight the importance of considering the impacts of metal pollution when assessing the health of mangrove communities and their ecological resilience to climate change.

TALK

# Translating ecological models into genomic simulations: a spatially-explicit reconstruction of muskox genomic data responding to climate change and human hunting.

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Investigating the effects of environmental changes on species distributions has been the central focus of biogeography studies over the past 50 years. This has been usually done by employing ecological niche models (ENMs), which use the climatic and environmental conditions in sites of occurrence of a species to find correlates in geographic space and time. Recently, advancements in modelling techniques allow to explicitly simulate population dynamics responding to global change drivers, incorporating ENMs into spatially-explicit population models (SEPMs). Given that the ecological processes that shape species distributions also determine patterns of genomic diversity, translating these spatially- and process-explicit ecological models into genomic simulations might provide new opportunities to better understand evolutionary processes in space and time, determine the genomic signatures of extinction and resilience, and directly evaluate the effects of environmental changes on the genome. In this study, we have developed a pipeline for translating process-explicit ecological models into spatio-temporal genomic simulations. Using muskox as a case-study, we used the ‘paleopop’ R package to simulate 21,000 years of population growth, extirpation, and dispersal responding to changes in environmentally suitable conditions and hunting by humans. Model outputs are gridded maps of simulated population abundances, which were used as input in the ‘slendr’ R toolkit to simulate muskox genomic data. Thus, we can directly sample genomes across space and time, for any model generated under the SEPM framework. This will allow us to determine how well process-explicit ecological models match (ancient or present-day) genetic data from a target organism, and potentially refine SEPM model fitting by matching real genomic data to simulated genomic data, in combination with ecological data matching. Our framework provides a way forwards towards genomically-informed process-explicit analyses of species distributions, to ultimately unveil the impacts of environmental changes at multiple biodiversity levels.

TALK

# Implementation of physiological performance in Species Distribution Models to assess the vulnerability of amphibians to climate change.

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One of the main consequences of the ongoing global warming is the shift of the geographical ranges of natural populations, which are forced to move to areas where their climatic requirements are now met. The main aim of the present study was to try and predict the future distribution of three Italian anuran species (*Rana italica, Bufo bufo, Bufotes viridis*) with different ecological and biological characteristics. We first quantified the thermal performance curves through laboratory trials. We then combined these estimates of their thermal tolerance with species distribution models to visualise and predict their current and future climate vulnerability, respectively. Our results suggest that species with broader physiological plasticity are predicted to achieve wider distributions than species with narrower physiological plasticity. In fact, when populations are unable to track their climate envelope across space as temperatures increase, their persistence depends on their ability to adapt to the new thermal environment. Hybrid models linking physiology and ecology showed to enhance the power of our predictions. Understanding and linking the ecological and physiological requirements of the study species resulted in a more accurate evaluation of future environmental drivers for their distribution and, ultimately, an effective conservation strategy. We observed, however, that the prevention of massive decline, and even extinction, of populations that combine limited physiology with specific ecological needs might not be viable.

TALK

# MicroRNA-mediated responses to environmental stress in marine microalgae

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MiRNAs are small non-coding RNAs (~20nt) that regulates the expression of genes by interacting with target transcripts. They can act in a pleiotropic but coherent manner to respond to different external stimuli. Nowadays, marine organisms are constantly exposed to environmental changes driven by anthropogenic impact, which cause severe events, e.g. shift in salinity of the waters, or eutrophication events with depletion of nutrients, or presence of chemical pollutants. However, organisms can survive to these conditions by acclimating to them. Indeed, miRNAs activity can give plasticity to organisms by participating to the gene regulatory networks, mediating the responses to environmental factors. For these reasons, we have identified and characterized miRNAs in the diatom *Phaeodactylum tricornutum* under three environmental stress conditions: nitrogen depletion, metal contamination, low salinity. We have sequenced their RNAs by SmallRNA-seq and predicted miRNAs bioinformatically using the tools miRDeep2 and miRDP-P2. Then we have identified their targets by using miRanda and psRNATarget. We have found about 70 novel miRNAs, for which we have analysed the differential expression in the tested conditions. Regarding their predicted targets, we have analysed the pathways which they belong by using ShinyGO. Interestingly, we identified many targets included in the photosynthesis regulation, which is a first response to the absence of essential nutrients and accumulation of oxidative stress, that could indicate a rearrangement of the cell physiology as a response to the environmental stress. Data validation by Stemloop-qPCR and by transcriptomic approach are ongoing. Validated observations will be included in a database that will be of aid for the scientific community to further explore the world of gene regulatory networks in diatoms.

TALK

# Genomics of Lessepsian fish invaders: investigating the evolutionary dynamics behind the successful colonisation of the Mediterranean Sea

Francesco Giannelli[1], Samuele Broccardo[1], Ernesto Azzurro[2], Antonis Petrou[3], Marina Chiappi[2], Grigorios Skouradakis[4], Josephine Paris[1], Flávia A. Nitta Fernandes[1,5], Lorena Ancona[1], Tilman Alpermann[7], Mark Dimech[7], Emanuela Fanelli[1], Emiliano Trucchi[1]

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The exponential increase in biological invasions worldwide, driven by globalisation and climate change is listed among the five biggest threats to the Earth's biodiversity. This trend poses a significant threat to the conservation of endangered species and ecosystems worldwide. The Mediterranean Sea emerges as notably affected by this phenomenon. Since the opening of the Suez Canal, a considerable amount of alien species have been entering the Mediterranean from the Red Sea, and their spread has been favoured by the consequences of climate change. Focusing on two successful Lessepsian fish invaders: *Siganus rivulatus*, a long-standing invader and *Pterois miles* a newly established invader, we aim to investigate the trends of different types of genetic diversity during the colonisation of the Mediterranean Sea. Our first objective is to investigate how the different timing and speed of invasion have shaped genetic diversity distribution and genetic load accumulation in these two invasive species. We also know that these species are adapting to significantly lower temperature and salinity levels than those in which they usually thrive. Our goal is to understand whether these two species are using the same gene combinations to adapt to the new environmental conditions. Lastly, considering different possible climate change scenarios, we aim to develop reliable predictive models to infer the possible future trajectories of these invasions, taking into account how non-neutral diversity may influence the invasive potential of these species. Preliminary results reveal a dramatic decline in genetic diversity moving from the source population to the northern bound of the invasive range with the Mediterranean population representing just a small portion of the total diversity present in the Red Sea suggesting that the colonisation of the Mediterranean is attributable to a single and rapid invasive event.

TALK

# Ocean Acidification will improve the response capacity of *Posidonia oceanica* against sea warming

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Ocean acidification (OA) and seawater warming are the main drivers of marine ecosystem changes. The dominant coastal ecosystem in the Mediterranean Sea is the *Posidonia oceanica* meadows, the only marine plant species endemic to this sea and strongly threatened by climate change. Although isolated effects of OA and temperature on seagrass performance have been explored, there are only a few studies concerning their combined effect on adult plants. In the present study, *P. oceanica* seedlings were grown under OA conditions for six months and then exposed to anomalous seawater warming. Phenotypic traits such as photo-physiology, antioxidant capacity, and energetic metabolism were analyzed and integrated with transcriptomic data using a weighted gene co-expression network approach (WGCNA) to explore possible interactive effects between these major climate change-associated stressors. Under controlled CO2 levels, anomalous high temperatures stressed *P. oceanica* seedlings by altering their photosynthetic and respiratory rates with the potential to erode their health and energy status. However, when seedlings were grown under OA conditions they were able to regulate the transcriptomic machinery to counteract the effects of heat stress by regulating their metabolic rates and enhancing their antioxidant defense. Elevated CO2 concentrations, rather than producing apparent stress to *P. oceanica* seedlings, provide them with greater “resources” to cope with heat stress conditions, which is reasonable since seagrasses tend to be carbon-limited under current CO2 conditions. Increased CO2 availability in future oceans could make *P. oceanica* meadows more productive and resilient to the ongoing climate change, changing the pessimistic view on the fate of this valuable ecosystem.

TALK

# Facing climate warming: life history traits and transcriptomic response in a pan-Antarctic tardigrade species

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Antarctic terrestrial life is mostly restricted to the small ice-free areas of the continent (<0.5 %) where animal communities are dominated by soil microarthropods and meiofaunal organisms (e.g., nematodes, rotifers and tardigrades). Increasingly warmer summers and more frequent warming events are leading to the transformation, damage, and degradation of the Antarctic ecosystems. *Acutuncus antarcticus*, the most abundant and common tardigrade in Antarctica, inhabits freshwater ponds, terrestrial soils, mosses, and lichens. Its suitability for laboratory rearing [4] makes it a model organism for studying the impact of rising temperatures resulting from global warming on Antarctic terrestrial communities. Life history traits and fitness of this species were analyzed by rearing specimens at 5°C and 15°C. Additionally, the first transcriptome analysis on *A. antarcticus* was performed on specimens exposed to increasing temperature (5°C, 10°C, 15°C, and 20°C) to identify differentially expressed genes under short- (1 day) and long-term (15 days) heat stress. Data on specimens reared at 5°C show an extended lifespan, a delayed sexual maturation, and an increased egg production, with reduced hatching rates compared to specimens reared at 15°C, whereas the fitness decreases in the second generation across both rearing temperatures. Heating induces significant transcriptomic changes, especially during the short-term exposure, suggesting alterations in mitochondrial activity, oxido-reductive processes, and the involvement in heat stress response of two tardigrade intrinsically disordered protein genes, typically associated with tardigrade desiccation. Nevertheless, transcriptional changes are very low during long-term exposure, suggesting that short-term variations may result from an acclimation response. Present data point out that *A. antarcticus* could be able to withstand increasing temperature over time, including future scenarios resulting from global climate change.

POSTER

Eligible for Best Poster Prize

# Genome plasticity in tiger mosquitoes: biological significance and relevance for pest management

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Mosquitoes (family Culicidae) are among the most relevant species for human health, being the primary vectors for pathogenic arboviruses, nematodes and protozoa. Several members of the family have become invasive in Europe due to human-mediated introductions from their typically subtropical native ranges. Despite the often-severe bottlenecks associated with the invasive process, these species, such as *Aedes albopictus* in Italy, demonstrate a remarkable ability to rapidly adapt to a wide range of environmental conditions. This phenomenon, often referred to as the "genetic paradox of invasive species", has been previously observed in other organisms, with transposable elements (TEs) hypothesized to play a role as mutational agents, allowing such rapid adaptations. This is particularly relevant for *A. albopictus*, where more than half of its genome is constituted by TEs. Here, we present a multidisciplinary approach aimed at elucidating this process by: (a) studying the TE landscape across the entire Culicidae family and on a population level scale; (b) investigating the role of transposons in the adaptive potential and, therefore, invasiveness of *A. albopictus* across the Italian peninsula. We are currently compiling a comprehensive dataset of whole genome sequencing from publicly available resources covering most of the Culicidae diversity. This dataset will be used to estimate the TE content and its evolutionary dynamics through the clade using a reads-based approach. At the same time, we are setting up an experimental design including lab-reared and natural populations sampled along temperature gradients across Italy to test the hypothesis that TEs are mobilized under (thermal) stress. Overall, these complementary analyses have the potential to provide novel insights into mosquito genome evolution and invasiveness.

POSTER

# A multidisciplinary research approach reveals a significant biological impact of global warming on the Italian freshwater crab *Potamon fluviatile* (Brachyura: Potamidae)

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Global warming significantly impacts ecosystems and ectotherms associated with freshwater habitats showed to be particularly vulnerable to climate change. In such scenario, our aim was to utilize behavioral and molecular techniques to study the impact of present and projected water temperatures on the Italian freshwater crab, *Potamon fluviatile*, which is classified as 'Near Threatened' by the IUCN. First, we investigated the species' thermal preferences by placing females and males in arenas containing three pools with water at 24°C, 28°C and 32°C. The crabs were individually recorded along three independent trials, during which we noted the time spent in each pool. Females preferred to stay at 28°C, while males spent more time at 24°C. Both sexes tended to avoid the pool at 32°C. We then investigated the intraspecific agonistic behaviors of adult males reared at different temperature regimes (22°C, 26°C and 30°C). After the exposure period, pairs of males of similar size were placed in an arena and allowed to interact for 15 minutes. We recorded the types of attacks, ritualistic behaviors, fight duration and attack latency. The exposure to high temperatures increased the duration of fights and the aggressiveness of dominant crabs. It also affected the type and length of interactions. Overall, our results show that the projected warming of the water of Italian streams will affect the biology of *P. fluviatile* and that this impact will be sex-specific. Further insights on such impacts are expected by our ongoing experiment on the gene expression in crabs exposed to different thermal regimes, which will be briefly described.

POSTER

Eligible for Best Poster Prize

# Population Genomics of the Endangered Butterfly *Hipparchia sbordonii* in the Pontine Islands

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The butterfly species *Hipparchia sbordonii*, endemic to the Pontine Islands in the Tyrrhenian Sea, is currently classified as endangered. Understanding the genetic makeup of this species is crucial for developing effective conservation strategies. This study focuses on the population genomics of *Hipparchia sbordonii*, aiming to assess genetic diversity, population structure, and genetic load, providing insights into its evolutionary dynamics and conservation needs. We collected specimens from the different islands of the archipelago, museum specimens, and additional specimens from the sister species *Hipparchia semele* in mainland Italy to perform whole-genome sequencing. By analyzing these genomic data, we estimated levels of genetic diversity within and between populations. Our findings revealed significant genetic differentiation among the island populations, likely due to limited gene flow and historical isolation. This genetic structuring underscores the importance of treating each island population as a distinct conservation unit. Furthermore, we examined the genetic load within these populations, identifying deleterious alleles that may impact the species' viability. Our analysis indicated a high genetic load, which could be a consequence of inbreeding and small population sizes. This genetic burden poses a significant risk to the long-term survival of *Hipparchia sbordonii*, as it may reduce fitness and adaptive potential. Overall, our study highlights the importance of genomic approaches in understanding the evolutionary and conservation biology of endangered species. The genetic insights gained from this research provide a foundation for informed conservation strategies aimed at preserving the unique genetic heritage of *Hipparchia sbordonii*.

POSTER

# Laboratory exposure to different thermal regimes shows a strong impact of global warming on the early life stages of the Italian great crested newt, *Triturus carnifex* (Amphibia: Urodela)

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Climate change has been shown to strongly affect the survival of early life stages of ectotherms. Indeed, tadpoles and larvae represent the most vulnerable life stages in amphibians, an animal group particularly impacted by global warming. Adverse thermal conditions experienced in the early life stages affect molecular responses critical for survival during development and adulthood. On these bases, our research aimed to investigate how prolonged thermal exposure can influence the early stages of the Italian great crested newt, *Triturus carnifex*, an urodele amphibian considered ‘Vulnerable’ in the IUCN red list. Adults *T. carnifex* of both sexes in their reproductive phase were sampled and allowed to mate under controlled conditions in the laboratory. A subset of the eggs produced was collected and randomly exposed to 13 °C, 18 °C - the optimal temperature for the correct development of this species - , 23 °C and 28 °C. Various features of the development of offspring were monitored at each thermal regime, until stages 43-44 (complete absorption of the yolk and active feeding phases). At the control temperature of 18°C, 46% of the eggs hatched after 20 days and 80% of them reached the 43-44 stage after 29 days. Similar results were obtained at 13°C, with 100% of offspring reaching the final stage after 35 days. A strong effect of higher temperatures was observed on hatching rates, length of development and survival of the offspring, with a total of 25% and 8% of the eggs hatched at 23 °C and 28 °C, respectively. At the end of the above tests, 3 larvae for each thermal regime were sacrificed for RNA extraction and subsequent analysis of the entire transcriptome, which is still in progress. Our results show a strong impact of global warming on the early life stages of the Italian great crested newt and call for further studies aimed at understanding the resilience to thermal stress of this vulnerable species.

POSTER

# Exploring Presence Absence Variation in mussels to infer species and adaptation in the Mytilus species complex

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It has been established that mussels possess an open pangenome, characterised by numerous hemizygous regions associated with structural variation, which can be subject to presence/absence variation (PAV) phenomena among individuals according to parental haplotypes. Such genomic regions are often associated with protein-coding genes, which can have significant effects on the phenotype of individuals. We have previously shown that dispensable genes are enriched in functions related to survival and immune responses, suggesting a possible contribution to local adaptation. However, until now, studies of PAV in mussels have only been conducted on *Mytilus galloprovincialis*, without taking into consideration the other inter-fertile species of the Mytilus complex, despite the well-documented occurrence of introgression. In our study, we extend PAV analyses to *M. edulis*, *M. trossulus*, and *M. chilensis*, categorising dispensable genes as softcore, shell, and cloud, and introduce the new category of hardcore genes, which are invariably shared by all species in the complex. The observed PAV patterns allow for very clear discrimination of individuals belonging to the four species, and identify mussels with mixed ancestry in a manner similar to SNP data. This further highlights the presence of highly diagnostic dispensable genes that are strongly associated with each species, but nearly absent in all others. Unlike SNPs, PAV data can be more easily associated with potential phenotypic functions and traits. This connection allows for a faster assessment of the evolutionary and adaptive implications of the loss or maintenance of dispensable gene clusters across populations. Although some technical challenges remain - including the lack of a true reference pangenome for all species, the difficulty in handling long reads, and the lack of genomic data for Southern hemisphere species - the results of this study suggest that PAV gene data could effectively complement SNPs in population genetics studies on mussels.

POSTER

Eligible for Best Poster Prize

# Acclimation in the invasive arboviral vector *Aedes albopictus*

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Acclimation is the physiological process through which organisms adjust to changes in their environment, mostly temperature, over a short period, enhancing their performance and survival. Hot-blooded organisms (endotherms), such as birds and mammals, maintain a constant internal body temperature regardless of external conditions. Their acclimation strategies are aimed to produce or conserve heat, like thermogenesis, blood flux regulation and sweating. Cold-blooded organisms (ectotherms), such as fishes or insects, depend on environmental temperature to modulate their physiology, thus shifts in temperature like those associated with current global changes are expected to greatly impact their biology, distribution and phenology. However, the physiological strategies used by ectotherms to cope with environmental temperature changes are not extensively known. Among the ectotherms, mosquitoes represent a threat for global health, and as global temperature rises, the habitats suitable for mosquito breeding and adult survival expand, leading to the geographic spread of species such as the Asian tiger mosquito, *Aedes albopictus*, a major vector of arthropod-borne diseases like Dengue fever, Zika, and Chikungunya. In this work, we aimed to identify the acclimation signatures of *Aedes albopictus* using a comprehensive approach including fitness, physiological and transcriptional analyses. We reared mosquitoes under standard laboratory rearing conditions (28 °C) and a warm thermal regime (32 °C) for one generation to let them acclimate to the new thermal regime. Then, we investigated the fitness of warm-acclimated mosquitoes with that of mosquitoes reared under standard conditions and compared the transcriptional response of these females at emergence day. We observed warm-acclimated *Ae. albopictus* mosquitoes have a faster larval developmental time, a higher reproductive output, shorter lifespan, and depleted energy reserves. We further observed extensive transcriptional changes with 956 upregulated and 428 downregulated genes in comparison to mosquitoes maintained at standard conditions. These results highlight the complex acclimation response of *Ae. albopictus* mosquitoes.

INVITED LECTURE

# All light, everywhere? A glimpse on the many roles of non-visual photoreceptors

Kristin Tessmar-Raible

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Our work focusses on the impact of solar and lunar light on aquatic organisms. On the one hand we focus on the molecular mechanisms of moon-controlled timing systems and their interactions with the circadian clock. In many marine organisms, like the bristle worm *Platynereis dumerillii*, moonlight sets the phase of an endogenous monthly oscillator. Some recent questions we have been tackling are: How can different worms across a population reliably synchronize to the same moon phase? How is lunar timing influencing daily timing? Can the study of molecular timing mechanisms of marine bristle worms help to understand some of the scientifically reported, but “just weird” correlations between human physiological/behavioral rhythms and the lunar cycle? On the other hand, we study photoreceptors that function under solar light (i.e. daylight). The latter uncovered unexpected roles of genes encoding light-sensitive molecules in processes such as the orchestration of animal life span, growth, cell divisions and cell differentiation trajectories. Given the large number of light-receptive genes whose biological roles are not yet understood both in invertebrates and vertebrates, these results emphasize that there are likely many unexpected unknowns on the role of light for animals. Our work emphasizes that the effects of artificial illumination on organisms are likely underestimated and that ideas, such as blocking solar radiation to cope with global warming, pose risks to ecosystems we do not even understand.

TALK

# Adapting to a rotating world: the molecular regulation of circadian clock in echinoderm larvae

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Most living organisms on Earth have evolved circadian clocks to synchronize their physiology and behavior to the daily alternation of light-dark cycles. These sophisticated adaptation systems work through a series of interconnected transcriptional-translational feedback loops driving periodic oscillations in mRNA and protein levels. The majority of animal circadian clocks is driven by a conserved group of genes including positive regulators (Clock and Cycle/Bmal) and negative regulators (Period, Timeless and Cry). Exploiting the available genomic and transcriptomic databases for 14 species belonging to the Ambulacraria clade, we identified homologs of all the canonical animal clock genes with the exception of Period, a key component of the negative loop in both protostome and deuterostome circadian oscillators. This gene loss was further validated by distant homology searches and phylogenetic analyses, suggesting that the Ambulacraria core clock architecture differs from other bilaterian circadian clocks. We then explored the spatial expression pattern of the conserved clock regulators in the sea urchin *Paracentrotus lividus* and the sea star *Patiria miniata* larvae, and discovered that different larval territories are employed for circadian regulation in the two echinoderm species. Finally, the ongoing analysis of the diel transcriptomes of *P. lividus* larvae coupled with physiological measurements will provide a detailed picture of echinoderm larvae adaptation to light-dark cycles and will lay the foundation for the identification of the negative components of the echinoderm clock. Our study explores the transcriptional and physiological dynamics of circadian rhythms in non-chordate deuterostomes and aims to shed light on the evolution and diversification of fundamental adaptation mechanisms to environmental periodicities in animals.

TALK

# Loss-of-function of a photoreceptor extends animal growth and lifespan

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Light is one of the environmental factors most affected by human impact on Earth. In animals it is sensed by opsins and cryptochromes expressed in various cells in addition to the eyes. Yet, although most animals possess more photoreceptors than humans, little is known about their roles outside rhythmic processes. Here we show that in the marine bristleworm *Platynereis dumerilii*, a loss-of-function mutation in the light-receptive cryptochrome (l-cry) significantly extents overall lifespan and increases adult size, a phenotype mimicked by wild-type reared in constant darkness. Quantitative transcriptomics of immature and premature l-cry mutant vs wild-type adult worms revealed hormonal players crucial for invertebrate and vertebrate sexual development and reproduction. These include nr0b, the common ortholog of DAX-1(NR0B1) and SHP (NR0B2), absent in arthropod lineages. Depending on moon-phase, nr0b is up or down regulated in l-cry mutants. Matching the complex regulation, loss of nr0b function partially recapitulates l-cry phenotypes. Molecularly, Platynereis nr0b affects steroidogenic and other endocrine pathways, nuclear receptor signaling, and transcription factor orthologs, involved in developmental, reproductive, and timing processes in other organisms. Our study reveals profound effects of light on animal lifespan, likely at least in part via similarities in the endocrine dynamics underlying sexual maturation and reproduction in annelids and vertebrates.

TALK

# Responses to light pollution: investigations on the physiology and behaviour of zebrafish at different developmental stages

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One of the widespread consequences of human activities is the presence of Artificial Light at Night (ALAN). ALAN disrupts the typical light-dark cycle under which most organisms have evolved, causing remarkable neurobehavioural alterations in terrestrial species. However, these effects are mostly unexplored in aquatic species, despite ALAN is common along freshwater and coastal marine environments. To fill this gap, we exposed adult and embryo zebrafish in mesocosms simulating ALAN conditions to investigate its impacts on behaviour, cognition and locomotor rhythmicity across developmental stages. After the treatment, the zebrafish were subjected to a characterisation of behaviour (open-field test, scototaxis and sociability), cognition (visual and spatial), and the circadian clock. Interestingly, zebrafish activity and lateralisation were affected by ALAN only at the larval stage. Furthermore, ALAN affected simple learning in larvae, but not in adults, who were instead affected in more complex cognitive functions. In a second group of adult specimens, we studied the circadian clock and found that most ALAN-treated adult zebrafish did not display daily patterns of behavioural activity and had disturbed photic entrainment. Brain transcriptomic analyses during the 24h revealed significant alterations in the expression of clock and clock-related genes after ALAN exposure. Particularly, we found alteration in the daily expression pattern of circadian clock (per1 and cry1), clock-controlled (aanat2) and melanopsin genes. Alterations in daily rhythmicity were also observed in zebrafish larvae exposed to ALAN condition for the first 9 days of life. Overall, our research confirms that ALAN significantly affects most aspects of zebrafish behaviour and cognition. Additionally, it showed that this impact varies depending on the developmental stage, suggesting age-specific effects of light pollution on biodiversity.

TALK

# Conservation of cis-regulatory syntax underlying deuterostome gastrulation

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Throughout embryonic development, the shaping of the functional and morphological characteristics of embryos are orchestrated by an intricate interaction between transcription factors and cis-regulatory elements. In this study, we conducted a comprehensive analysis of deuterostome cis-regulatory landscapes during gastrulation, focusing on four paradigmatic species: the echinoderm *Strongylocentrotus purpuratus*, the cephalochordate *Branchiostoma lanceolatum*, the urochordate *Ciona intestinalis* and the vertebrate *Danio rerio*. Our approach involves comparative computational analysis of ATAC-seq datasets to explore the genome-wide blueprint of conserved transcription factor binding motifs underlying gastrulation. We identified a core set of conserved DNA binding motifs, associated with 62 known transcription factors, indicating the remarkable conservation of the gastrulation regulatory landscape across deuterostomes. Our findings offer valuable insights into the evolutionary molecular dynamics of embryonic development, shedding light on conserved regulatory subprogrammes and providing a comprehensive perspective on the conservation and divergence of gene regulation underlying the gastrulation process.

TALK

# Sensory control of sea urchin larva development and growth: interplay between light and food availability

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Living in a complex and constantly changing environment, the planktotrophic sea urchin larva has evolved mechanisms of response to cope with these conditions. An example is the phenotypic plasticity in response to food availability: when food is abundant, plutei develop shorter arms to save nutrient storages while when food is scarce they develop longer arms to collect it more efficiently. This response was shown to be mediated by a dopaminergic signalling. Nonetheless, the sensory nervous system controlling such a response remains largely uncharacterized. In this study, we applied a multidisciplinary approach to characterize and compare the larva sensory nervous system of two sea urchin species: *S. purpuratus* (from the Pacific Ocean) and Paracentrotus lividus (from the Mediterranean Sea). More in detail, we combined immunostaining, whole mount fluorescent in situ hybridization, phenotype observation and gene perturbation experiments with scRNA-seq data. We especially focused on a group of cells which are both sensory (expressing the Go-Opsin3.2) and neurosecretory (secreting the neuropeptide TRH) in *S. purpuratus* that we found to control arm growth. Our data suggest that the TRHergic cell type has been rewired in *P. lividus* and provide new evidence on how the sea urchin larvae sensory-neurosecretory system collects and integrates information from a variety of environmental cues (especially food and light) in order to control larval growth and to understand how such processes evolved on a relatively short evolutionary scale.

TALK

# Cell Diversity in Sponges

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Sponges are a diverse group of animals and represent one of the earliest animal lineages. They offer unique insights into the origin of key animal-specific traits as sponges lack complex structures such as muscle, neurons and gut. There are four classes of sponges, Homoscleromorpha, Calcarea, Hexactinellida and Demosponges, that diverged during the pre-Cambrian period. Our current understanding of the cell diversity is restricted to demosponges (i.e., *Spongilla lacustris* and *Amphimedon queenslandica*). To address this gap in our understanding of sponges, we focused on the calcarean sponge, *Sycon ciliatum*. We used a combination of FACS and 10X Genomics to profile the transcriptome of ~30,000 cells and produced the first cell atlas of a calcarean sponge. Moreover, we performed a cross-species comparison with the existing atlases of Spongilla and Amphimedon. Our results clarify the evolution and diversity of cell types in sponges.

TALK

# Pleiotropy drives the cryptic persistance of male reproduction in asexual stick insects.

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Our understanding of how traits evolve along the Tree of Life is advancing rapidly beyond the simplistic paradigm of novel trait emergence and irreversible trait loss. A closer look reveals that some complex adaptations in extant species can be explained as the reinstatement of traits that were once lost in their ancestors. Yet, theory predicts that after the loss of a trait, the molecular machinery underlying its phenotypic expression should decay. To bridge this knowledge gap, we tested the hypotheses that (i) the molecular ground plan of a lost trait could persist due to pleiotropic effects on other traits and that (ii) the gene co-expression network architecture could constrain individual gene expression. Our testing ground was the Bacillus stick insect species complex, which includes close relatives that are either bisexual or parthenogenetic. After identifying genes expressed in male reproductive tissues in a bisexual species, we investigated their gene co-expression network structure in two parthenogenetic species. We found that gene co-expression patterns within the male gonads were partially preserved in parthenogens. Furthermore, parthenogens did not show relaxed selection on genes associated with male gonads in the bisexual species. As these genes were mostly expressed in female gonads, this preservation could be driven by pleiotropic interactions and an ongoing role in female reproduction. Connectivity within the network also played a key role, with highly connected—and more pleiotropic—genes within male gonads also having a gonad-biased expression in parthenogens. Our findings provide novel insight into the mechanisms that could underlie the production of rare males in parthenogenetic lineages; more generally, they provide an example of the cryptic persistence of a lost trait's molecular architecture, driven by gene pleiotropy on other traits and within their co-expression network.

TALK

# Unraveling the genome of *Congeria kusceri*: evolutionary insights into subterranean adaptations in a unique cave-dwelling bivalve

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By using state-of-the-art methodologies, we obtained a chromosome-scale genome assembly of the cave-dwelling bivalve mollusk *Congeria kusceri*. The genome, which consists of 17 chromosomes and includes 60342 annotated protein coding genes, reveals substantial differences from its closest available surface relative, *Dreissena polymorpha*, displaying a significantly slower evolutionary rate, which is in line with its longer generation time, as well as with its living fossil status. Nevertheless, despite its critical conservation status and small population size, *C. kusceri* retains high heterozygosity, which may be a relevant factor for future conservation efforts. The comparative analysis of the genomes of Congeria and other heteroconch bivalves offered a remarkable opportunity to investigate the evolutionary mechanisms underlying the unique traits linked with its adaptation to the subterranean environment. These encompass the loss of pigmentation and light sensing, metabolic alterations, the acquisition of a peculiar reproductive strategy and a remarkable longevity. A thorough comprehension of these genomic attributes is vital for uncovering the mechanisms that have enabled *C. kusceri* to thrive in the challenging underground environment, as well as to protect this species from the threats posed by habitat degradation. This resource can help understanding convergent evolution processes, which in this case for example involve the parallel loss or pseudogenization of genes involved in the regulation of circadian rhythm and light sensing between Congeria and deep-sea bivalves. Moreover, since Congeria is one of the very few extant cave-dwelling bivalves, the analysis of its genome might shed light on the existence of common genetic mechanisms underpinning cave adaptations across different phyla.

TALK

# The mechanisms of bivalve Doubly Uniparental Inheritance of mitochondria: from comparative molecular evolution to early embryo transcriptomic data

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Bivalves represent powerful testing grounds for evolutionary convergence, due to the independent occurrence of traits such as freshwater colonization and extended longevity. Another interesting and overlooked feature that occurs in several bivalves is the Doubly Uniparental Inheritance of mitochondria (DUI). In these species, two mitogenomes are differentially transmitted across generations: oocytes transmit only female-type mitogenomes, while spermatozoa transmit exclusively male-type ones. The co-presence of these two evolutionarily independent mitochondrial lineages is the only stable exception to strict maternal inheritance in Metazoa, and the coevolutionary pathways of mitochondrial and nuclear genomes, as well as the mechanisms involved in the selective inheritance, are far from being understood. In our project we investigated omics-derived data from 36 bivalves covering most representative families and including 6 occurrences of DUI. Comparing nearly 9000 single-copy orthologues, we identified a set of ~200 genes showing stronger purifying selection in DUI species and enriched for evolutionary correlations with mitochondrial proteins. Many of these genes were associated to microtubule-based transport processes, and we identified some candidates that might be involved in the peculiar pattern of mitochondrial aggregation that occurs specifically during male embryogenesis. Crossing these results with RNA-Seq data of eggs we obtained from *Mytilus galloprovincialis* females that produced strong sex-biased progenies (up to >90% same-sex offspring), allow us to focus on more promising candidates that might be involved in the sex-specific mitochondrial inheritance. In our still ongoing project, these candidate genes and their associated pathways will be investigated in situ and in the light of a Single-Cell RNA-Seq time series of early embryo stages we will perform in the next mussel reproductive season.

TALK

# Rhythmic contractions of the yolk of goldfish, domesticated ornamental fish with diverse dorsoventral patterning traits

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Rhythms play an important role in the precise spatiotemporal regulation of biological processes during embryonic development. The extent to which these rhythms contribute to the emergence of novel phenotypes remains understudied. We here investigate the rhythmic contractions of the yolk during early development of the goldfish *Carassius auratus*. We quantify these contractions and record robust and persistent rhythmic yolk movements that are not seen in closely-related species (carp *Cyprinus carpio* and zebrafish *Danio rerio*). We report that yolk contractions are an intrinsic emergent property of the egg. These contractions do not require sperm entry / fertilization nor cell division, and they notably emerge at a precise time - suggesting that goldfish eggs are able to measure elapsed time from what we infer to be egg activation. As the yolk itself is known to confer critical cues for early dorsoventral (DV) patterning of teleost embryos, we hypothesize that its contractions in goldfish may influence the patterning process of this species. Indeed, we find that embryos in conditions that result in ventralized phenotypes (i.e. goldfish embryos acutely treated with microtubule-depolymerizing drug nocodazole and embryos of the twin-tail goldfish strain Oranda) display altered yolk contraction dynamics (i.e. faster and/or stronger contractions). We aim to uncover whether the yolk contractions happening during early development of domesticated goldfish are the licensing process which explain the variety of novel DV patterning phenotypes naturally-observed in this species (e.g. twin-tail and dorsal-finless strains) and which are instead not found among closely-related species (e.g. carp, also from the same ecological niche and similarly subject to domestication) whose yolks do not contract.

TALK

# Thyroid hormones trigger stomach differentiation during metamorphosis of the common clownfish (*Amphiprion ocellaris*)

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Many marine animals have a biphasic life history: larvae live in the open ocean, while juveniles move to coastal environments and exploit a completely different ecological niche. The transition between these two life stages is also often associated with a radical change in diet, and requires an overall transformation of the larva. Such a process is the process of metamorphosis, which, in vertebrates, is under the control of thyroid hormones. Using the clownfish as an accessible laboratory model system for the study of teleost metamorphosis, and here focusing on the gastrointestinal tract, we show that metamorphosis brings about the anatomical appearance of a new organ, the stomach, and the corresponding glandular differentiation of what was the distal oesophagus mucosa, independently of dietary change. At the transcriptional level, we show that these anatomical changes provide metamorphosing larvae with the new capability for acid digestion. Moreover, through pharmacological perturbation of the thyroid hormone signalling axis we show that the hormone triiodothyronine (T3) controls stomach metamorphosis, and we resolve the deiodinase1 (dio1) - thyroid hormone receptor a (thra) pathway as its most likely signalling axis based on spatio-temporal transcription patterns of thyroid hormone signalling components. Overall, these results confirm stomach emergence as a key feature of metamorphosis in gastric altricial teleost species, and characterise such a process from the organ level down to its molecular mechanisms. We further present an extension of this model to selected teleost families with atypical gastric developmental patterns. These findings inform not only the aquaculture and larval rearing of these species, but also the still poorly understood process of foregut and stomach development in teleost species.

POSTER

# Larval development of *Holothuria tubulosa*, a new tractable system for evo-devo

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A challenge for Evo-Devo is to expand the breadth of organisms used to investigate how animal diversity has evolved through changes in developmental gene regulatory networks. As a phylum of the sister group to chordates, echinoderms extensively contributed to our knowledge of embryonic patterning, organ development and cell-type evolution. Among echinoderms, holothuroids have all the features of valuable experimental systems, e.g.: ease of collection from the field, abundance of eggs that develop into synchronous cultures of optically transparent embryos and larvae, but lack reproducible methods to culture embryos in laboratory conditions. As a result, the molecular regulation of their development is still mostly unexplored. To fill this gap, we established the sea cucumber *Holothuria tubulosa* as a new experimental system for cell and developmental biology. This species is highly abundant in the Mediterranean Sea, a draft genome has been recently published and embryos develop through planktotrophic larvae. We set up a reproducible protocol to obtain gametes and culture *H. tubulosa* embryos up to the juvenile stage. Combining immunohistochemistry, high resolution microscopy and drug treatments, we highlighted critical cellular and developmental features enabling us to explore the evolution of animal cell types. We defined skeleton and nervous system development and discovered a new biofluorescent cell type of unknown function whose pigment has specific spectral properties and high biotechnological potential. This work represents a step forward in our understanding of holothuroids development and establishes *H. tubulosa* as an emerging and easily accessible experimental system for Evo-Devo studies and beyond.

POSTER

Eligible for Best Poster Prize

# Exploring the evolution of sex determination mechanism in Pseudo-nitzschia diatoms

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Sexual reproduction is a conserved feature in eukaryotes, and various sex-determination systems have emerged across different lineages. Within unicellular organisms, the opposite sexes are referred to as Mating Types (MT). In 2018, the first, and currently unique, diatom MT-determining gene was identified in the marine species *Pseudo-nitzschia multistriata*. The gene MRP3 was found to define the MT+, regulating the expression of four downstream genes: MRP1 and MRP2, upregulated in MT+, and MRM1 and MRM2 in MT-. We questioned if all diatoms share this mechanism. Our focus is on the diverse genus Pseudo-nitzschia, specifically on two species from distant clades on its phylogenetic tree. Several strains with both MTs have been isolated for *P. arenysensis* and *P. calliantha* from the Gulf of Naples (Italy, Mediterranean Sea). We plan to sequence their genomes and employ a transcriptomic approach to compare their gene expression profiles. The genome sequencing, with long-reads technology, for both species is ongoing. In *P. arenysensis*, 46 genes were found to be Differentially Expressed Genes (DEGs) between opposite MTs. Notably MRP3 is differentially expressed, suggesting that its role as master regulator of sex determination is conserved; this hypothesis will be proven by MRP3 overexpression and tagging. At least two other unannotated genes, DN374 and DN7000, have been discovered as MT+ specific, of which the DN700 protein is predicted to be localized in the chloroplast. MRM2 expression does not appear to be dependent on the MT, while the conservation of the MRP2 role in MT+ is confirmed. For *P. calliantha* the RNA-seq is completed and the DEG analysis will be elaborated as soon as the reference genome has been sequenced. This project will enhance our understanding of a fundamental biological process, also in terms of evolution. The comprehension of this mechanism in diatoms is crucial for understanding genetic diversity, in turn relevant to explain the adaptation capacity to environmental changes.

POSTER

# Selective Pressure on Deep Sea Octopod Light Sensing Molecules

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Characterized by high intelligence and a diversified lifestyle, octopods represent one of the most distinctive animal groups. Species within this group are distributed across pelagic, deep-sea, and littoral environments. Octopods are renowned as active predators, relying on a multitude of senses to locate and capture their prey. Notably, their exceptional visual acuity, facilitated by well-developed camera eyes, and remarkable camouflage abilities underscore the importance of studying their sophisticated light-sensing molecular machinery. By integrating bioinformatics with molecular biology approaches, we elucidated the expression patterns and molecular evolution of opsins—the primary photoreceptive molecules in Metazoa—across various octopod species inhabiting the Gulf of Naples (Italy). Our analyses revealed that the ancestral octopod possessed five opsins: two r-opsins, one peropsin, one retinochrome, one c-opsin, and one xenopsin, with the latter undergoing independent duplication in *Argonauta argo*. Additionally, we performed in silico selection pressure analysis (PAML) on visual r-opsin1 (Octopus rhodopsin homologues) and the photoisomerase retinochrome in littoral (*Octopus vulgaris*, *Callistoctopus macropus*, *Eledone moschata*), deep-sea (*Eledone cirrhosa*, *Pteroctopus tetracirrhus*, *Scaeurgus unicirrhus*), and pelagic species (*A. argo*), and analysed the sites selected by mapping them on the 3D structure of the proteins.Our results indicate that, while retinochrome shows positively selected sites in all the deep-sea species, possibly requiring higher energy wavelengths (blue shift) to perform photoisomerization, r-opsin1 under selection only in *P. tetracirrhus*. The analysis of selected sites points to a shift toward the green, matching the light emissions of benthic bioluminescent prey.

POSTER

# Custom-Designed Orchid Bait Sets and their Phylogenetic and Ecological applications

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The Orchidaceae family, with its vast diversity and intricate evolutionary history, poses challenges to researchers in phylogenetic and ecological studies. We addressed these challenges by developing two custom-designed orchid bait sets, 20KNuclearOrchidbaits and 400PlastidOrchibaits, utilizing nuclear and plastid genomes/transcripts from orchid species. Our comprehensive in silico evaluation revealed the efficacy of these bait sets across orchid species of all subfamilies of Orchidaceae. The 20KNuclearOrchidbaits had a successful hybridization across 38 species, averaging 14,325 baits (ranging from 7,108 to 19,555 baits). The 400PlastidOrchibaits showed high performance across 635 orchid plastomes, with approximately 356 hybridizing baits (ranging from 147 to 400 baits). We experimentally tested these new orchid bait sets in a phylogenetic analysis of Catasetum and Ophrys species. In both cases, we recovered a high number of parsimony Informative Sites (PIS) with a strong phylogenetic signal. These bait sets also facilitate accurate pollen identification: SNPs identified in the phylogenetic analysis were also used to identify anonymous pollen samples, overcoming the limitations of traditional pollen DNA barcoding methods and potentially enhancing ecological studies on orchid-pollinator interactions. In conclusion, our orchid bait sets may represent a versatile tool with broad applications in phylogenetic and ecological studies.

POSTER

Eligible for Best Poster Prize

# An evolutionary and multi-disciplinary approach to study sex determination and germline development in bivalves

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Bivalves are vital in many aspects of human economy and societies, with the aquaculture industry largely benefiting from the harvest of key species. Yet, the current understanding of sex-determining processes is essentially unknown, limiting any advancement in farming practices. Therefore, establishing a comprehensive framework to design solid evolutionary studies for future research on bivalve sex determination is of primary importance. As a matter of fact, despite the growing amount of publicly-available sequencing data, our knowledge is still very fragmented and limited to few model species. Here, we leveraged both comparative genomics and mRNA in-situ HCR to advance our understanding of bivalve sex determination. In particular, we extensively mined genomic and transcriptomic resources to identify genes belonging to the dmrt, sox, and fox gene families, whose components have been already appointed as related to sexual processes. Combining phylogenetics and molecular evolution analysis in a broad spectrum of the bivalve taxonomic diversity, we found that genes likely involved in the male sex-determination cascade are private to bivalves, and show a higher amino acid divergence than genes not involved in sexual processes. Furthermore, we explored the temporal and spatial transcription patterns of three sex-determination related genes and the germline marker Vasa in the early stages of *Mytilus galloprovincialis* embryogenesis, documenting a germline specification process that differs from the one observed in *Crassostrea gigas*. Overall, these findings suggest that future research should leverage more extensively the use of multidisciplinary approaches to study sex determination in bivalves, by also adopting an evo-devo perspective. As a matter of fact, single model systems cannot represent alone the diversity of groups of organisms as diverse as bivalves.

POSTER

Eligible for Best Poster Prize

# Parthenogenesis in Timema stick insects: repeated evolution or ancestral potential?

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Traits that evolve independently multiple times are crucial for understanding the mechanisms of evolution, as they are often thought to result from similar or identical mutations in unrelated lineages. The Timema genus comprises five obligate parthenogenetic species of stick insects, each in sister relationship with a bisexually reproducing species. The current literature states bisexuality as the ancestral state for this clade, with parthenogenesis having evolved five times, independently. In this study, we investigate the alternative hypothesis of parthenogenesis either evolving once or being the ancestral condition in each of the lineages. The first hypothesis proposes that parthenogenesis evolved just once, and its taxonomic distribution could be explained by hemiplasy, a process leading to discordance between trait and species trees caused by incomplete lineage sorting or introgression. We tested this using both inference-based approaches and by identifying hemiplastic candidate genes, which we characterized using gene ontology analyses. The second hypothesis suggests that parthenogenesis was the ancestral state, with bisexuality being the derived one. We explored this hypothesis through two lines of research: the different probability of relative rate of shifts in sexual strategies from parthenogenetic to bisexual, and proteomic analyses showing fewer changes in parthenogenetic taxa compared to their bisexual sister, suggesting bisexuality as a derived trait. Our findings illustrate how traits appearing as evolutionary novelties may be built on ancestral ground, and highlight the potential for misinterpreting evolution due to misleading inferences.

POSTER

Eligible for Best Poster Prize

# Exploring the evolution of pancreatic-like cell types in sea urchins at a single-cell resolution

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The digestion of food sources is an essential function for animals' survival. It relies on the production of digestive enzymes and of hormones involved in metabolic homeostasis. In vertebrates, the organ responsible for controlling such processes is the pancreas. The formation of a specific pancreatic organ is a vertebrate innovation but pancreatic-like cell types have been found outside of vertebrates, together with a highly conserved expression of canonical pancreatic genes such as Pdx-1 (pancreatic and duodenal homeobox-1). Pdx-1 in humans guides pancreas formation, β-cells differentiation, and subsequently controls insulin production in the latter, resulting as a causal factor in some types of diabetes. Despite the evident conservation of Pdx-1 expression in gut cells of most deuterostomes, the evolutionary origin of pancreatic cells is completely unknown, and it is not clear when the regulatory link between Pdx-1 and insulin emerged. Sea urchins are representative of non-chordate deuterostomes, and it has been previously shown that *Strongylocentrotus purpuratus* larvae possess several pancreatic-like cell types, one of which appears to be morphologically and molecularly homologous to its mammalian counterpart. Here, we explore for the first time the pancreatic-like cell type composition of the Mediterranean sea urchin *Paracentrotus lividus* larva under a differential feeding regime and compare it with other sea urchin species using single-cell transcriptomics.

# Modern systematics: improving the way biodiversity is captured, explored and understood

INVITED LECTURE

## New models to measure biodiversity using artificial intelligence

Daniele Silvestro

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Throughout the long evolutionary history of life, species of all kingdoms have undergone staggering diversification and faced countless environmental changes and extinction events. Since the great majority of species that lived on Earth have since gone extinct, it is difficult to infer how biodiversity dynamics unfolded over millions of years and yet crucial to understand the fundamental evolutionary processes driving them. Today, with over a million species threatened with extinction, biodiversity is facing unprecedented challenges, urging the need for conservation policies that maximize its protection and sustain its manifold contributions to people. Here we present a suite of new supervised and unsupervised models with applications in systematics and in evolutionary and conservation biology. Specifically, we show how deep learning models can be used to infer biodiversity dynamics in deep time and present a new semi-supervised framework to improve phylogenetic inference. Finally, we introduce a reinforcement learning framework to optimize biodiversity conservation policies. Coupling artificial intelligence with evolutionary models holds great promise for advancing our understanding of biodiversity and its evolution across time scales, and for improving biodiversity conservation action in a rapidly changing world.

TALK

# Opsin diversity in TARA

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The TARA Ocean expedition travelled all around the globe for over four years to collect plankton samples and characterize them from ecological, morphological, and molecular points of view. With over 272 sampling locations, reaching depths up to 1000 meters, it represents one of the most taxonomically rich metagenomic and metatranscriptomic datasets in the world. We mined these datasets to look for opsins, G-protein-coupled photoreceptors essential for animal vision. They are generally categorized into four main groups: r-opsins, c-opsins, xenopsins, and Group-4 opsins. Using a complete pipeline involving the combination of molecular clustering methods, protein annotations, and phylogenetic analyses, we characterized the different opsins at both the taxonomic (phylum) and gene subfamily levels. We found that the majority of the opsins belong to arthropod and vertebrate visual opsins. We further identified sequences representing all the major opsin groups, including cnidarians, acoelomates, arthropods, mollusks, annelids, chaetognaths, rotifers, and echinoderms. Our phylogenetic analysis shows that R-opsins are more basal, with C-opsins having a sister relationship with Xenopsins, and no additional new opsin family was discovered, supporting previous phylogenetic studies. Using the TARA Ocean dataset, we were able to provide novel opsin sequences from various taxonomic groups and locations, which can be explored in further research.

TALK

# Unveiling pseudo-cryptic speciation in storm petrels: insights from genomics, morphometrics, and bioacoustics

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Due to the reliance on morphological differences in species delimitation, morphological stasis hinders the classification of reproductively isolated lineages as different species. By combining genomic, morphometric and bioacoustic data, we investigated cryptic speciation throughout the range of the European storm petrel (*Hydrobates pelagicus*), a small and nocturnal pelagic seabird of a family that exhibits strong morphological stasis (family Hydrobatidae). We uncover high genetic differentiation between Atlantic and Mediterranean storm petrels and estimate their divergence time at ~75 kya, coinciding with a sudden sea level reduction. We also identified three finer-scale genetic clusters within each of these basins, a pattern mirrored by morphometric and acoustic differentiation. We detected ancestral gene flow but no signs of recent gene flow between Atlantic and Mediterranean storm petrels; demographic inference suggested that gene flow could have ceased due to range-wide population declines at the beginning of the Holocene. Our results support the presence of two pseudo-cryptic species, the Atlantic storm petrel (*H. pelagicus*) and the Mediterranean storm petrel (*H. melitensis*), and an additional conservation unit, the Atlantic storm petrel from the Canary Islands. Our findings highlight the need for a thorough re-assessment of the range-wide conservation status of this species complex, including the Macaronesian conservation unit, especially because both Mediterranean and Macaronesian populations are small and show uncertain population trends.

TALK

# Heterogeneous relationship between rates of speciation and rates of phenotypic evolution in a hyperdiverse fish clade

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Some groups of organisms achieve spectacular richness in both species and morphological diversity whereas others, in the same evolutionary time, do not. Indeed, evolutionary rates often vary among clades and lineages, with fast-evolving groups accumulating new species, phenotypic diversity or both faster than slow-evolving groups. An open question is whether rates of speciation and phenotypic evolution are correlated. Classical theory focussing on adaptive divergence suggests that these rates should be associated (i.e., fast-speciating lineages are also fast in evolving phenotypic changes) and has received some empirical support. However, other empirical studies have failed to find an association between speciation rates and rates of phenotypic evolution. Here, we move past previous efforts by using several phenotypic traits - including principled projections of multivariate data. As focal clade we use wrasses, a hyper diverse clade of marine fish comprising the families Labridae, Scaridae (parrotfish) and Odacidae. Using a new dated phylogeny, as well as advanced geometric morphometric and statistical techniques, we find: 1. substantial rate variation within this clade in both speciation and phenotypic evolution, 2. that whether a robust association between speciation rates and rates of phenotypic evolution is recovered depends on the phenotypic trait considered, 3. in the case of wrasses, the relationship between evolutionary rates is significant only for a specific aspect of body shape which may be interpreted in terms of genetically-driven phenotypic integration, 4. that the strength of the relationship between rates of speciation and rates of phenotypic evolution, when present, varies between fast-evolving and slow-evolving lineages. In addition to providing a deeper understanding of evolutionary patterns in wrasses, these results have broader implications on the way we address the general question of whether different rates are associated with each other.

TALK

# Hidden lineages in "well-studied" butterflies: the case of *Erebia euryale* and *E. ligea* in the South-Eastern Alps

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Our understanding of the biological diversity is still incomplete for many clades of the "tree of life". Even in well-studied groups, the species delimitation in use is often challenged by new studies leveraging genomic and phenotypic data, as well as by new statistical approaches and theoretical frameworks for the analysis and integration of these data. European butterflies represent one such group, for which many recent studies unveiled previously overlooked species-level lineages and intraspecific structure, or, conversely, extensive gene flow among species. The closely related *Erebia euryale* and *E. ligea*, for instance, are widespread in many European mountains and their intraspecific diversity has been recently revised exploiting both morphological and mitochondrial DNA data. Here we further investigate their taxonomy through modern genomic analyses. We sampled several populations of these butterflies across South-Eastern Alps and focusing on the Dolomiti Bellunesi, where both species and different subspecies are recorded. We obtained double-digest Restriction Associated DNA (ddRAD) sequences from 70 specimens. Bayesian clustering, Bayesian estimates of recent migration, and networks provide evidence for 3 lineages, with at least one putative hybrid between two of them, and apparently similar levels of gene flow. We also detected a fine-scale geographic structure within each lineage, partly matching current systematics. Our preliminary results suggest that even in apparently well-studied animals, like European butterflies, the taxonomy in use is only partially mirroring the actual evolutionary diversity. We are complementing this study integrating the genomic evidence here presented with the analysis of the phenotypic differentiation, including colour of wings and shape of copulatory organs.

TALK

# FAIRy tales of microendemism: disclosing dung beetle diversity and evolution in the Eastern Arc Mountains biodiversity hotspot (Tanzania) using integrative taxonomy and semantic technologies

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The ancient rainforest archipelago of the Eastern Arc Mountains (EAMs) of Tanzania and Kenya is one of the major biodiversity hotspots on the Earth, hosting an exceptional amount of endemic taxa. Unlike the more emblematic vertebrates and plants, the EAMs' arthropod fauna is still largely unexplored. We focused our study on dung beetles (Coleoptera: Scarabaeinae), a model group in ecology and evolutionary biology which offers a great potential for new discoveries in the EAMs. We investigate the diversity and evolution of five different dung beetle lineages using an integrative taxonomic approach which combines morphological and molecular species delimitation and phylogenetics. Additionally, we use these taxa as a test bench for tools to make the newly disclosed phenotypic information FAIR - Findable, Accessible, Interoperable and Reusable. Specifically, we implement Phenoscript, a new programming language that uses anatomical ontologies to create computer-parsable taxonomic descriptions, as well as nanopublications. Our results show the existence of many undescribed species in the EAMs and outline different speciation scenarios. The genera Amietina, Janssensantus and Onthophagus present a "one species per mountain block" endemism pattern, while the genus Grebennikovius has four distinct species that evolved microallopatrically within the forest of the Uluguru Mountains. Moreover, preliminary work on Tanzanolus hints at a complex morphological and molecular scenario of ongoing diversification, with one population having evolved brachyptery. Overall, EAMs dung beetle diversity appears to have been shaped by unique interplays between past rainforest fragmentation, local scale topography and species characteristics such as flightlessness. Our work stresses the urgent need of further research to thoroughly understand the diversity and evolution of dung beetles in the EAMs, which is essential for their conservation. Additionally, our pioneering approach with Phenoscript to craft semantic descriptions aims at providing a new standard for FAIR taxonomic papers.

TALK

# The evolution of corallivory in the Coralliophilinae (Gastropoda: Muricidae)

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Host-parasite relationships provide very useful models to study adaptive processes. We investigated the interactions between parasitic marine gastropods, the Coralliophilinae Chenu, 1859, and their cnidarian hosts. Coralliophilinae is a muricid subfamily of caenogastropods that feed on octocorals (Anthozoa: Octocorallia) and hexacorals (Anthozoa: Hexacorallia). Limited information is available regarding the phylogenetic relationships of this corallivorous mollusc lineage, as well as, the degree of specificity of the parasite-host interactions. To investigate coralliophiline/coral relationships in the context of their evolution, we generated the largest molecular dataset so far, comprising two mitochondrial (COI and 16S-rDNA) and one nuclear (ITS2) gene fragments from 586 coralliophiline specimens collected all over the world, from depths ranging from 0 to 1000 metres. To identify the coral hosts of the coralliophiline species, we integrated literature data with an empirical approach. For the former, each coralliophiline species present in the tree was related to the cnidarians identified in literature at the lowest possible taxonomic level. New data were then produced: when the cnidarian host sample was available, morphological identification was conducted alongside the amplification of the 16S-rDNA molecular marker; when the whole gastropod body was available, host DNA was amplified by barcoding the snail stomach content. Sequences were then matched against a reference database. The obtained molecular phylogeny of the Coralliophilinae revealed the existence of several complexes of cryptic species; moreover, some of the genera as traditionally conceived were not confirmed as monophyletic. The occurrence of repeated shifts to deep waters were confirmed. Concerning trophic ecology, our findings indicated that the subfamily's ancestor evolved in shallow waters in association with Scleractinia. Across the evolutionary history of Coralliophilinae, multiple host shifts to other cnidarian orders were observed, not correlated with changes in the depth range.

TALK

# The phylogeny of 3000 Saccharomyces cerevisiae strains inferred by metagenomic approach

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Phylogenomic investigation of micro-eukaryotes is generally based on a series of time-consuming steps involving isolation from the primary matrix, clone sequencing, genome assembly, and the identification of single-copy orthologs. Metagenomic approaches can be used to reconstruct eukaryotic MAG genomes directly from metagenomes, but their efficacy in automatically inferring strain-level phylogeny has not yet been thoroughly explored. Here, we used the bioBakery pipeline to infer a large phylogeny for S. cerevisiae from a set of nearly 3,000 samples comprising both sequenced clones and shotgun metagenomes from various environments. The pipeline returned an alignment of circa 1,000 species-specific S. cerevisiae markers automatically reconstructed by StrainPhlan. Maximum likelihood analysis of this dataset recovered a well-structured, highly supported yeast phylogeny. The tree is in accordance with previous phylogenies of reference-based alignments from solely clone sequencing, confirming the efficacy of the metagenomic-based approach. Furthermore, the large sample size and the addition of strains from metagenomes provide an updated phylogeny of S. cerevisiae with an unprecedented resolution, identifying new source-specific clades from, for example, kefirs, palms and olives, and revealing that some widely recognised clades cannot be solely defined by their environmental source nor by their geographical origin. From a methodological point of view, our results confirm that metagenomic approaches can successfully be used for the intraspecific (strain level) analysis of micro-fungi such as yeasts.

TALK

# Using Environmental DNA to characterize deep-sea benthic annelid communities: a case study in the western Mediterranean Sea

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Deep-sea ecosystems represent one of the most extensive biomes in the biosphere. Due to their vastness, inaccessibility, and the high costs of sampling campaigns, their biodiversity is poorly known, and the majority of the species occurring in these environments is expected to be undescribed. Annelids, being the most important group in terms of biomass and species number, are particularly interesting for studies in deep Mediterranean environments. Despite their significant ecological role, they are scarcely studied from the taxonomic point of view, as several ecological studies rely on taxonomic sufficiency, avoiding species-level identification and do not integrate morphological information with molecular data. However, studies employing integrated taxonomy and environmental DNA (eDNA) metabarcoding techniques, revealed surprisingly diverse annelid communities with a higher degree of endemicity than expected. In this study DNA was extracted from 64 sediment samples collected from various deep-sea environments in the western Mediterranean: a bathyal plain (Gulf of Salerno), two seamounts (Marsili and Palinuro), and a canyon (Dohrn Canyon). In the same sites, polychaetes individuals were sampled using traditional methods (grab sampling, sorting, and identification), barcoded, and their sequences were used to build a custom reference library for subsequent eDNA analyses. The extracted DNA was amplified using two markers (16S rDNA and COI), using primers specifically optimised for polychaetes. The outputs of these analyses will be useful to describe the structure of benthic communities in these unique deep-sea habitats with high taxonomic resolution, thanks to the reference sequence library. The detailed description of annelid assemblages and the entire associated benthic community at the finest possible taxonomic level, using integrative taxonomy and eDNA in the deep Mediterranean Sea, contributes to a more accurate assessment of their diversity and rate of endemicity. This approach is also essential for obtaining reliable estimates of the impact of anthropogenic drivers on this poorly studied ecosystem.

TALK

# *T. Panzhihuanense* long reads genome assembly and genome-scale divergence time estimation reveal Cenozoic diversification of truffles followed by lineage-specific expansion of Gyspy LTRs

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Ascomycetes of the genus Tuber, also known as true truffles, are symbiotic filamentous fungi characterized by the formation of hypogeous fruiting bodies and distinctive flavors that aid in their dispersion by animals, making them food delicacies known for centuries. Due to their economic importance, multiple truffle genomes have been sequenced so far, highlighting peculiar genomic features such as a remarkably high proportion of transposable elements (TEs) and low gene redundancy. However, these studies are affected by the high fragmentation of the assemblies and the lack of a solid timeframe for truffle diversification. Here, we solved these gaps through a high-quality, near-complete genome assembly of the Chinese *Tuber panzhihuanense* together with the first genome-scale fossil-calibrated time tree for Pezizales. Thanks to a high contig N50 of 7 Mb, we were able to explore previously hidden genomic features of truffles, such as rDNA cluster organization, the structure of an intron-rich mitochondrial genome of 302 Kb, TEs genomic distribution, and syntenic relationships with their sister clade Morchellaceae. We additionally revealed that Tuber diversification started ~50 million years ago during the Cenozoic, concurrent with the rise and expansion of angiosperms and the major diversification of mammals. Following the diversification of Tuberaceae, different Gypsy LTR-retrotransposons have independently expanded across various truffle lineages, leading to a commonly dominant but highly diverse Gypsy genomic content. We provide evidence that these TE amplifications may have affected syntenic relationships with Morchellaceae, as well as gene family evolution, potentially increasing gene-family turnover rates in the clade. Overall, this study highlights the concurrent expansion of extant truffles within the context of the angiosperm terrestrial revolution and provides novel insights into the tempo and mode of TE evolution in an economically important fungal clade.

POSTER

# The RIBICALM Project: development of a tool for the determination of Sweet chestnut (*Castanea sativa* Mill.) varieties using machine learning.

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The sweet chestnut tree is present around the entire Lake Como (Lario) where for centuries it has constituted an important productive resource for local communities and where it provides an important environmental protection service. This plant, typical of fresh and humid mountain territories, is increasingly subject to desiccation due to drought, to diseases that attack it when weakened and to the overall alteration of the environment. The RIBICALM Project aims at characterising the Chestnut varieties of Lario and Valtellina, to provide the various mountain communities with a better knowledge of the territorial resources. To this purpose, we are sampling three specimens for each “variety” identified on an historical basis, or, more often, by oral tradition. All trees will be genotyped at 15 SSR loci and their genotype used for several of the supervised learning approaches used in data mining. The goal is to obtain a probabilistic attribution to one of the varieties already defined in a previous phase. The genetic and morphological results, integrated with historical and cultural data, will allow us to plan correct conservation strategies both by identifying varieties that are not yet known and by highlighting genetic relationships with already known varieties.

POSTER

# The “wicked” problem of the threat to biodiversity posed by invasive insect species

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The accelerating pace of commercial and social globalisation, fostered by climate change, is creating unprecedented opportunities for species to move into new areas outside their natural range. Insects are the most abundant and species-rich animal taxon and unsurprisingly they are frequently introduced beyond their native range. Invasive insect species are a considerable threat to human health and food security and can cause significant economic losses due to reduced crop yields and high management costs. Moreover, when exotic insect species integrate into an ecosystem, new interactions arise with both native species and other invaders, leading to disruption of ecosystem services and a high impact on native biodiversity, causing in some cases the displacement or even extinction of local species. In recent years, two polyphagous hemipteran species, *Halyomorpha halys* (Pentatomidae) and *Aleurocanthus spiniferus* (Aleyrodidae), have been introduced in Italy, causing in some regions considerable economic damage. Different approaches have been used to allow their rapid identification, to assess their genetic variability and to study their relationships with native species. In addition, different alien parasitoid species of these exotic invaders have been found in Italian regions, opening new opportunities for their biological control, but also posing new challenges due to their potential impact on native biodiversity. Current results highlight the importance of pursuing different approaches for collecting and producing data that will benefit national and local management of invasive species.

POSTER

Eligible for Best Poster Prize

# cRanial: an r shiny app based on three-dimensional models to assist in mammal skulls identification

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In various fields of biological sciences, the integration of technological tools has revolutionized traditional methodologies, particularly in the study of morphological structures. Three-dimensional (3D) models have become essential tools in disciplines such as palaeontology, anthropology, and zoology and are increasingly used in educational contexts, including biodiversity, museology, and ecology. Here, we introduce an innovative Shiny application designed to facilitate the analysis of mammal skulls through interactive 3D models and landmark-based morphometrics. This Shiny app assists in performing exploratory biodiversity analyses and recognizing mammal skulls. The tool employs a comprehensive dataset containing high-resolution 3D models of mammal skulls, annotated landmarks, and phylogenetic trees, offering a robust resource for the scientific community and the educational sector. By employing an intuitive and user-friendly graphical user interface, the app enables users with uncertainties about skull recognition to identify them using interactive figures and landmarks starting from a 3D model uploaded by users. Beyond research applications, the app significantly contributes to museology by aiding in the cataloguing and digital archiving of osteological collections. Additionally, its utility in educational contexts provides a hands-on learning experience for students exploring vertebrate anatomy and evolutionary biology. The application’s potential to enhance both scientific research and education underscores its value as a multifaceted tool in the study of mammalian morphology and macroevolutionary biology.

POSTER

# The Moorish gecko (*Tarantola mauritanica*): model to explore ecological speciation

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The Moorish gecko (*Tarantola mauritanica*) is a common medium size lizard, widely distributed in the western Mediterranean basin. Geckos are described as primarily nocturnal, and exhibit a heterogeneous colour pattern ranging from whitish-grey to brownish-black, depending on ecological and physiological conditions. We deepened information on these poorly investigated correlations using field, molecular and experimental data. We used a multidisciplinary approach (i.e. microsatellite analysis, capture/marking/release, skin reflectance measurement, melanin concentration) to provide strong evidence supporting the hypothesis that the Moorish gecko is experiencing an incipient ecological speciation within two sympatric populations. Indeed, the dark and pale geckos show temporal (day-night) and spatial (tree trunk- wall) segregations, keeping still a weak gene flow each other. Using a molecular clock model, based on complete mithogenome sequencing, we have found an evolutionary split between the two morphotypes dated close to the introduction of fire as artificial light by Homo sapiens in the Mediterranean basin. The multifaceted evidence we gathered suggests the potential influence of human-controlled fire in facilitating the temporal niche partitioning in Moorish geckos, since it represents an environmental novelty associated to a new, important foraging niche at night. However, some questions still remained unanswered, and future researches are needed to explore aspects of this intriguing scenario, describing for the first time a synanthropy driven by light.

POSTER

# High-Resolution Melting (HRM) Analysis as a Tool for Cetaceans Species Identification in the Mediterranean Sea

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Species identification of Mediterranean marine mammals mainly relies on passive acoustic monitoring or on the analysis of morphological features, whose efficiency will depend essentially on the animal behavior and environmental conditions. The use of genetic information for cetacean species identification is limited, therefore, new approaches that can contribute to better monitoring of stranded species are needed. In that context, the proposed High Resolution Melting (HRM) method offers a new approach to identifying marine mammal individuals at the species level. We selected the seven most common cetacean species housing the Mediterranean area, and we designed specific mtDNA primers in the most hypervariable regions. The HRM primer combinations revealed a success rate of 100% in terms of discrimination for all species included in this study, and HRM analysis resulted in ranges of accuracy of 80% and 90% in terms of precision. This approach is not only less time-consuming and easy to handle, but it also allows for easy discrimination and can produce results quickly. This suggests that HRM analysis could be an effective alternative tool in species identification, particularly in stranded marine mammals where the high degree of degradation often leads to the loss of many morphological features or in the recognition of putative hybrids. Moreover, the HRM method could also be a valuable tool in the field, aiding in detecting the illegal trade of CITES-listed species. It would also provide a greater degree of ease for zoologists, veterinarians, biologists, and other field-related professionals to interpret results when effectively classifying cetaceans. However, it is crucial to note that further studies with environmental samples and a broader geographical scope involving distinct populations are necessary.

POSTER

Eligible for Best Poster Prize

# Fertilization in pig, not a simple reverse domestication

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Feralization is an evolutionary trajectory occurring in various domesticated animal populations, such as chickens, dogs, horses, pigs and sheeps. All feral populations living in the wild must express adaptations aimed at making functional a phenotype initially shaped by humans according to their preferences. Numerous and diversified adaptation strategies are described among various feral species. In particular, swine (*Sus scrofa*) are an excellent model system to investigate feralization since i) they currently exhibit both domestic (pig) and ancestral (wild boar) forms, which are able to cross-breed; ii) feralization often arises through a soft-artificial selection, which involves a combination of artificial and natural selection. We deepened information about feralization in Sardinian free-living pigs, which revealed fascinating adaptations that enable them to cope with natural selection (e.g. recovery of sense of smell through variations in morphology and gene expression in the olfactory mucosa). We speculate that traditional Sardinian pastoralism drives an unexpected return to the wild, creating a unique genetic pattern, different both from the domestic and wild forms. In particular, pastoralism appears to reduce gene flow between wild boar and free-living pig, while favouring specifically some adaptations to survive in wild (i.e. foraging for food independently), however preserving some clearly domestic desirable phenotypes (i.e. mildness, fatness, spotted coat colour). Our findings contribute to the ongoing discussion on the current European policies for the management of feral pigs, the effective conservation actions for preserving diversity in suidae forms, and their consequent impact on biodiversity linked to their adaptive strategies.

POSTER

# A DNA barcode library for mangrove crabs and gastropods of East Africa

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Mangroves are tropical and subtropical intertidal forests present on sheltered coasts across the world. There has been a significant decline in mangrove forest cover in recent decades. Africa hosts 20% of the world's mangroves, 26% of which are found on its eastern coast. They are home to a unique faunal community, dominated by brachyuran crabs and gastropods, which play critical ecological roles within the ecosystem. Assessing the overall macro-invertebrate diversity is thus essential to develop effective management and conservation plans for mangrove forests. Such endeavour is hindered, however, by the difficulty of species identification due to the presence of a high number of cryptic and pseudocryptic species and phylogenetically related ones. In this study, we provide a verified reference DNA barcode library for crabs and molluscs inhabiting East African mangroves. Using PacBio technology, we obtained the partial sequences of the mtDNA cytochrome oxidase subunit I gene from about 260 morphologically-identified specimens from the Natural History Museum of the University of Florence. Barcode sequences were then used to delineate Molecular Operational Taxonomic Units (MOTUs) and measure the barcode gaps among congeneric species. This study provides the first reference barcode library for East African mangrove-associated macrobenthic fauna and represents a critical tool to 1) avoiding long-lasting species identification errors known for this geographical area, 2) solving the discrepancies between morphological and genetic identifications present in various public databases, and 3) clarifying the diversity of the extant communities of invertebrate resident fauna associated with one of the most pristine mangroves of the world, as recently assessed by the IUCN Red List of Ecosystem project.

POSTER

# Exploring the diversity of *S. cerevisiae* wine strains in spontaneous fermentations using (meta)genomics

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Modern winemakers commonly inoculate grape must with commercial *Saccharomyces cerevisiae* strains to obtain controlled fermentation and reproducible products. However, wine has been produced for thousands of years using spontaneous fermentations from wild strains, a practice that is experiencing a revival among small wine producers. Despite the widespread usage of such strains in the past, there is much to know about their ecology, evolution and functional potential. In particular we shall clarify the reciprocal affinities of these strains within the S. cerevisiae phylogeny, the degree of their genome diversity, their ecological origin (wine cellar or vineyard?), and their general impact on wine terroir. Here we sequenced the genome of 45 S. cerevisiae present in spontaneously fermented musts sampled across 24 Italian estates; we sequenced selected clones to avoid metagenomically assembled chimeras due to the co-presence of strains in must. These genomes will serve downstream oenological analyses; to plan conservation acts in a scenario of continuous climate change, we are maintaining the sequenced strains. We used an assembly-free metagenomic approach to reconstruct the strain-level phylogeny of our strains together with 500 available wine yeast strains sampled globally from both clones and metagenomes. Our phylogeny reveals unexpected high genetic diversity: although most strains are grouped in the well known “wine” clade, many others are scattered throughout the *S. cerevisae* phylogeny, in clades that were not previously known to include wine related strains.

POSTER

Eligible for Best Poster Prize

# Towards an Italian DNA barcode reference library for inland water fishes

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Freshwater ecosystems are affected by multiple anthropogenic stressors and are experiencing one of the highest rates of biodiversity decline among extant ecosystems. In this context, monitoring approaches based on DNA barcoding and metabarcoding represent powerful tools for planning effective conservation programs and guiding sustainable fisheries management. The success of these methods, however, depends on the availability of comprehensive and curated DNA barcode libraries. Here, we provide a sequence reference library based on the 12S rRNA gene for fish species known to inhabiting Italian inland waters. Our approach combines two complementary strategies to generate a comprehensive database covering the Vert01 and Tele02 12S rRNA gene fragments. First, we gathered existing sequences from major data repositories, including BOLD, NCBI, ENA, RNAcentral and MitoFish. Extensive data mining and validation were conducted to ensure the accuracy and completeness of the fragments. Second, we performed de novo sequencing using Illumina platform from museum voucher specimens belonging to species either not represented in these databases or for which high-quality sequences were not available. The obtained DNA barcode reference library represents a significant step forward in the identification and delimitation of fish species of Italian inland waters and in the development of monitoring programmes based on the environmental DNA (eDNA) approaches.

# An evolutionary approach to the biology and ecology of behaviour

INVITED LECTURE

## A comparative perspective on vocal communication and cognition in bats

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Bats are well suited for comparative studies because of the diverse set of sensory adaptations and social structures the >1450 species exhibit worldwide. In this talk, I explore bats’ communicative and cognitive skills from a comparative perspective. In the first part of my talk, I focus on vocal communication, specifically the information content encoded in bat vocalizations and the causes and consequences of vocal production learning. Vocalizing bats reveal a wealth of personal information such as individual ID, motivational status, regional origin, etc., and phylogenetically controlled analyses suggest a positive feedback loop between social and vocal complexity. In species capable of vocal production learning, the vocal domain has even more degrees of freedom. Vocal specialties or aberrations can be transmitted from generation to generation via social learning, and these vocal ‘traditions’ have fascinating evolutionary consequences. In the second part of my talk, I highlight new avenues for studying bat cognition across species. While social cognition is comparatively well studied in bats, physical cognition – i.e. how animals use and acquire information about the physical world – is not. I introduce new methods for studying physical cognition in bats and present first results on bats’ numerical competence and their understanding of object permanence.

TALK

# Swimmers or climbers? Eels' individual migratory strategies are explained by complex syndromes

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Individual variation is a crucial driver of adaptive evolution, yet it is often overlooked in studies of animal movement. We investigated the role of individual differences in one of the most intriguing examples of animal movement: the upriver migration of the highly endangered European eel. This migration poses significant challenges, including transitions between saltwater and freshwater environments and habitat alterations caused by human activities. By simulating a migratory setting in the laboratory, we identified distinct phenotypes among migrating glass eels. Some individuals exhibited a strong tendency to swim against a constant water flow, while others demonstrated a greater propensity to climb barriers. We then conducted a comprehensive characterization of the glass eels' biology, encompassing physiology, personality, cognition, and life history. The variation in these traits was significantly interconnected, even across different domains (e.g., physiology and cognition). When reducing this variance to primary axes, we found that they could predict the individuals' migratory types. Our study underscores the importance of variability among migrating individuals and its intrinsic relationship with multidomain trait variance. These aspects should be considered in conservation efforts for this endangered migratory species, as they likely contribute to its adaptability and resilience.

TALK

# Interspecific differences in behavioural responses of wild ungulates and mesocarnivores towards recolonising wolves

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The fear of apex predators can trigger changes in the behaviour of prey and smaller carnivores, in turn promoting adaptive responses in prey and predators. Major antipredator tactics involve temporal or spatial avoidance, but the nature of antipredator response is not ubiquitous. The novel exposure to a predator may stimulate rapid responses in prey and smaller carnivores, helping to understand antipredator behaviour. We studied behavioural responses of ungulates and mesocarnivores to the wolf *Canis lupus* in a protected area recently recolonised by this predator, i.e., the Maremma Regional Park (central Italy), through camera trapping (2017-2022) and diet analyses (2016-2023). The wolf settled permanently in the area since 2015 and based its diet mainly on wild ungulates (c. 75-80% of diet), with mesocarnivores being a negligible component of wolf food habits (<2%). Results suggested that prey responses were based on (i) temporal avoidance for the fallow deer *Dama dama*, that switched from crepuscular/nocturnal to diurnal activity, (ii) increased gregariousness for the wild boar *Sus scrofa*, (iii) a complex spatiotemporal avoidance mediated by nocturnal brightness, for the roe deer *Capreolus capreolus*. A strong spatiotemporal association between mesocarnivores and the wolf was found, with no support for avoidance and consistently with the 'dietary facilitation' hypothesis for the red fox *Vulpes vulpes*. During 2016-2023 the volume of fallow deer in the wolf diet decreased from 45% to 14%, whereas that of wild boar increased from 35% to 63%; prey densities did not show major changes. Results indicate (i) interspecific differences in antipredator responses, (ii) prey switching by the wolf driven by antipredator behaviour, (iii) that a diverse and rich prey community can reduce negative relationships between carnivores and can buffer predators against reduced prey accessibility because of antipredator responses, contributing to limit predator attraction to livestock.

TALK

# Diet comparison between two dwarf elephant species from the Pleistocene of Sicily thorough dental mesowear analysis

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We investigated the diet of two extinct dwarf insular elephant species, *Palaeoloxodon falconeri* and *Palaeoloxodon mnaidriensis*, from the Pleistocene of Sicily. The two species descended from a mainland ancestor, *P. antiquus*, which reached the island in at least two different dispersal events, differentiating respectively into *P. falconeri* about 800 thousands years ago and into *P. mnaidriensis* about 400 thousands years ago. We carried out a comparative dental mesowear analysis, by measuring the angles between enamel peaks and dentine valleys in the teeth lamellae of the two species. Through these measures it is possible to infer whether the diet included a higher percentage of more abrasive food (grazer behavior, associated with wider angles) or a less abrasive, soft food (browser behavior, associated with narrower angles). Diet in the two species was previously studied using microwear analysis, a technique that, however, can only provide information on the diet of the last few days, and thus it is strongly influenced by contingent factors related to the death time of the specimen. Mesowear, on the other hand, can reflect the diet across a time span of several weeks or even longer, thus providing more significant information. We analyzed 31 teeth (18 *P. mnaidriensis* and 13 *P. falconeri*) from the Paleontological Collection of the University of Padua (Museum of Nature and Humankind), collected in four sites rich in Pleistocene fauna: Grotta Boccadifalco (Palermo), Grotta Luparello (Palermo), Grotta dei Puntali (Carini) e Grotta ZÃ Minica (Capaci), all located in the Palermo province. Preliminary results show a prevalence of grazing in both species, with very wide mesowear angles. The diet in the two Sicilian species was even more grazing-oriented than that of their common mainland ancestor *P. antiquus*. This would confute the hypothesis that, due to the different ecological conditions that characterized Sicily during the Middle Pleistocene, the two species had adopted different diets.

TALK

# Fight or flight? Alternative mating strategies and the iconic male polymorphism of the European stag beetle

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The European stag beetle *Lucanus cervus* is a protected species and known by laymen for its charismatic appearance, characterized by a disproportionately large mandibles of males used in fights for females. Moreover, the size of males is subject to variation within populations. The existence of different male morphologies within a single species is associated with alternative mating strategies across different animal groups, offering textbook examples of evolutionary biology. In *L. cervus*, larger males are advantaged in fights and the fights occur through the aggregation of several individuals, while smaller males are more agile in flight. We present the first evidence of an alternative mating strategy for the smaller males: describing aggregations of flying males competing to catch flying females in mid-air. Flight aggregations occur earlier in the season and in sites with sparser canopy cover as compared to fight aggregations, but most importantly, smaller males tend to prevail as different abilities are rewarded. The male that succeeds in catching the female falls with her on the ground followed by few or no other males. The discovery of an alternative strategy offers a new perspective on the evolutionary interpretation of the iconic male polymorphism of *L. cervus*.

TALK

# The role of dietary omega-3 long chain poly-unsaturated fatty acids on cognition development and ecological performance in brown trout.

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Omega-3 long chain poly-unsaturated fatty acids (n-3 LC-PUFA) are necessary for survival and reproduction for many vertebrates including fishes. How dietary n-3 LC-PUFA influence brain development and animal cognition and behaviour in natural habitat remains unknown. Also, according to the speed-accuracy trade-off of animal cognition, animal personality and cognitive flexibility are linked together so that bold individuals have lower cognitive flexibility. Differences in cognitive and personality traits can influence the ecological performance of individuals. In this study, we tried to fill these gaps focusing on how dietary n-3 LC-PUFA affect brown trout (*Salmo trutta*) cognition and personality through behavioural scoring in laboratory conditions. We also searched for the correlation between personality and cognitive traits and fish resource acquisition after a rearing period in a semi-natural stream mesocosm, hence how the dietary treatment affects fish ecological performance, through stomach content analyses (biomass and taxonomic composition). Finally, we analyzed brain cellular composition and neurons number through the isotropic fractionator technique. We expected that fish which received an enriched diet in n-3 LC-PUFA, have a higher composition in brain cells and neurons and perform better at the cognitive test than the ones who received instead a depleted diet. We also hypothesized that these individuals have a slow personality type, which allows them to explore a natural habitat deeper than their fast counterpart, catching more prey and detecting also cryptic species. This results in a higher prey biomass and taxonomic diversity in their stomachs, and hence a better ecological performance.

TALK

# Female reproductive fluids: current insights, new approaches, and future perspectives

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Female Reproductive Fluids (hereafter FRFs, i.e. those mediums, arising from females, through which sperm must pass on their way to fertilize eggs) have historically received less attention than their male counterpart, seminal fluid, despite their emerging significance in regulating gametic interactions. Our research explores various aspects of the FRF's influence on gametic interactions in both externally and internally fertilizing fish species. Using the zebrafish, *Danio rerio*, we discovered that FRF enhances overall fertilization success, and promotes multiple paternity in the offspring by prolonging the time window available for eggs fertilization. Furthermore, we found that the change in sperm performance mediated by FRF can directly predict the change in paternity share between rival males from absence to presence of FRF. This pivotal result unequivocally identifies FRF as an indicator of female post-mating preference for specific males, underscoring its importance as a tool for assessing partner compatibility, with important implications for assisted reproductive techniques and conservation plans. We are exploring, in parallel, the possibility that FRF can mediate a choice towards a more genetically compatible partner (with the zebrafish), and/or towards specific male phenotypes, using as study system the grass goby, *Zosterisessor ophiocephalus*, a species with male alternative phenotypes. Research on this model uncovered a complex conflictual interaction between male and female reproductive fluids, that ultimately plays a central role in determining the outcomes of ejaculates competition. Finally, current research is focusing on the putative mechanisms of female allocation of FRF (in the grass goby), and the potential of FRF to act as a post-mating barrier against hybridization and/or inbreeding in the swordtails *Xiphophorus malinche* and *X. birchmanni*, closely related species prone to natural hybridization. In this presentation, I will elucidate the multifaceted impacts of FRF on reproductive processes and delineate some unresolved questions that could carry potential applications across diverse research fields.

TALK

# Not all neighbours stink. Responses to conspecific odours in species with different social structures.

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Sociality is a widespread strategy in the animal kingdom, but it often requires individuals to be able to identify and recognize group members from non-group members. Bats are highly social and show a wide variety of social structures. However, little is known about the role of olfaction in the evolution and maintenance of bat sociality. Here, we compare the behavioural responses to conspecific odours of two bat species with very similar ecology but different social organization: *Myotis bechsteinii* and *Myotis nattereri*. While *M. bechsteinii* is known to form strictly closed social groups, *M. nattereri* shows a higher inter-group connectivity. We presented two maternity colonies (one per species) with pairs of bat boxes containing conspecific faeces from either the same or a foreign colony. Using behavioural data collected with automatic RFID-loggers, we quantified and tested different behaviours related to roost exploration and roost occupation. Faeces' odour was highly relevant in roost exploration and occupation for both species, confirming that bats can perceive and respond to roost odour and faeces presence. However, the direction of the response was different in the two species. While *M. bechsteinii* females avoid boxes with cues of foreign conspecifics' presence, *M. nattereri* shows a clear preference for them. We show that bats' response to cues of foreign conspecifics can differ between species, and suggest that roost odour may play a relevant role in the maintenance of bats' diverse social structures.

POSTER

# Lizard from small island: the role of natural selection and the adaptive responses

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Lizards (*Podarcis sp.*) are highly adaptable to a variety of environments in both continental and insular areas. In particular, there are many cases of insular colonization both in large and small islands, often described as subspecies in order to chromatic variations. On islands, selective pressures are exacerbated by the inability of leaving the site (habitat tracking), and they are further amplified on small islands due to significant environmental stochasticity. This can lead to two possible outcomes: extinction or populations' adaptation through rapid plastic phenotypic variations. In this study, we provide empirical evidence of plastic adaptations in small island populations of Podarcis sp., showing a suite of morphological, behavioral, metabolic and physiological traits aimed to increase fitness. Specifically, ecological segregation and behavioral traits, including male-male interactions and reproductive efforts, seem to be typical of insular animals inclined to live intensely. These phenotypic variations, described in island populations compared to neighboring mainland populations, are related to the up-regulation of genes involved in metabolic and reproductive processes. Data collected from 21 small island in Tyrrhenian Sea support our evolutionary hypothesis: these lizards are more aggressive, bolder and more voracious than their mainland conspecifics, investing more resources in reproduction to face the challenges imposed by severe and unpredictable selective pressures on small islands.

POSTER

Eligible for Best Poster Prize

# Impact of nest density on dynamics of sexual selection in the Mediterranean damselfish *Chromis chromis*

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Pre- and post-mating episodes of sexual selection work in tandem to shape overall male reproductive success. Although there is strong evidence that various environmental, ecological and social factors can modify patterns of sexual selection, few studies have considered how such factors influence relationships between pre- and post-mating sexual selection and how much these relationships are dynamic in a changing environment. Here we investigate how variation in nest density affects dynamics of sexual selection in the damselfish *Chromis chromis*, an abundant and key species in the rocky coastal regions of the Mediterranean Sea where it acts as a vital conduit for nutrient transport from pelagic to benthic habitats. Through scuba visual census, behavioral observations, morphological measurements and physiological analyses on the ejaculate we performed a comprehensive investigation on male mating success and on the investment on pre- and post-mating behaviors and traits in relation to nest density and, thus, to potential competition for mating. In our study population, despite the high abundance of nesting sites, males chose to nest either solitary or in more crowded nest areas. This choice is not related to male size or condition index. Male size, however, positively influences mating success as larger males receive more eggs. Nest density does not influence clutch size, but at higher density nesting males have higher sperm quality and spend more time in guarding their clutches, suggesting a general higher investment rather than a trade-off between traits/behaviors. The higher investment necessary to face male-male competition at higher densities might be compensated by the advantage of a lower predation risk through group dilution effect. Overall, our preliminary results set the basis to deepen into the conditions and contexts that influence the dynamics of pre- and post-mating sexual selection and their interplay with other fitness components.

POSTER

Eligible for Best Poster Prize

# The effect of competition between the invasive blue crab and the native green crab on their diel activity: a multidisciplinary approach

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Organisms evolved biological rhythms to synchronize their physiology and behaviour to regularly fluctuating environmental parameters, both abiotic (photoperiod, temperature) and biotic (competition). The niche partitioning theory posits that sympatric competitors will evolve different temporal activity patterns to limit intra- and inter-specific interactions and coexist in the same environment. Biological invasions are among the main drivers of biodiversity loss, and they strongly impact coastal and estuarine habitats. The introduction of invasive species is known to impact the timing of activities and biotic relationships of native species. Conversely, both native and invasive species may adapt their temporal niches to the competitor's presence to maximise their fitness and survivorship. We investigated how intra- and inter-specific competition alters the daily activities of two euryhaline crabs, the native Mediterranean green crab (*Carcinus aestuarii*) and the invasive Atlantic blue crab (*Callinectes sapidus*). Both species are characterized by a high tolerance to variations in temperature, salinity, and air exposure, and are both keystone species in the trophic web of their native environments. Diurnal and nocturnal activities of the two species were videorecorded in mesocosms under control (no competitor), intra- and inter-specific competitive conditions. The time spent in the various activities and the feeding behaviours of focal crabs were recorded and analysed. To simulate competitive interactions, the crabs were placed in aquaria divided in two chambers by a perforated transparent screen to allow water to flow between the two chambers. Thus, crabs were able to chemically and visually perceive the competitors, without direct interactions. Transcriptomic analyses will be carried out on tissues extracted from a subset of the experimental specimens, in order to identify differential expression of genes up- and downregulated under intra- and interspecific competition.

POSTER

# The stick insect genus Bacillus and the influence of small mitochondrial highly transcribed RNAs (smithRNAs) on hybridization and speciation.

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The stick insect genus Bacillus exhibits intricate reproductive dynamics, with species such as *B. grandii*, *B. rossius*, and *B. atticus* demonstrating various modes of reproduction, including bisexual and facultative parthenogenetic forms. Hybridization among these species has led to the emergence of new hybrids, such as *B. whitei* and *B. lynceorum*. Mitochondrial DNA analysis has revealed asymmetrical hybridization events, where *B. rossius* consistently serves as the maternal parent. Recent research has focused on small mitochondrial highly transcribed RNAs (smithRNAs), a class of small non-coding RNAs encoded by the mitochondrial genome and involved in regulating nuclear gene expression. The distribution and characteristics of smithRNAs were examined across the parental Bacillus species. The results indicated a significant presence of smithRNAs in *B. grandii* and *B. atticus*, while *B. rossius*, the mitochondrial donor species for hybrids, exhibited minimal quantities of smithRNAs. These findings suggest a potential role for smithRNAs in cyto-nuclear interactions and the evolution of isolating mechanisms driving speciation within the Bacillus genus. This study highlights the importance of understanding the molecular mechanisms underlying hybridization and speciation processes, particularly in taxa exhibiting reticulate evolution. The Bacillus stick insects serve as an experimental model for exploring the complex relationships between sexual and clonal taxa, offering insights into the evolution of reproductive strategies and species boundaries. By examining the role of smithRNAs in hybridization, this research underscores the significance of mitochondrial-nuclear interactions in shaping the evolutionary trajectories of hybrid species. Overall, the research on Bacillus stick insects provides valuable insights into the molecular underpinnings of speciation and the evolution of reproductive strategies. The findings suggest that smithRNAs may play a crucial role in the cyto-nuclear interactions that drive the speciation processes within the Bacillus genus. Understanding these mechanisms can help elucidate the broader principles governing hybridization, speciation, and the maintenance of species boundaries in organisms with complex reproductive modes.

POSTER

# Darwin goes to the “El Farol” bar: investigating the emergence of efficiency and fairness in an evolutionary game

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Living organisms can establish an intricate network of mutually influencing relationships with their environment. In this study, a genetic algorithm is used in an attempt to model a complex adaptive system that addresses the El Farol Bar problem. The problem was introduced by W. Brian Arthur to illustrate the dynamics of decision-making under conditions of bounded rationality. It describes a toy ecosystem with two different microhabitats and a threshold for access to resources. The agents of our computational model are the mathematical objects known as finite automata (here “flibs”, as labelled by A.K. Dewdney) that respond to binary inputs from the environment based on their internal state and the transition table encoded in their chromosomes. The number of states of the flibs, which regulates the diversity of possible chromosomes, parameterizes the degree of complexity of the system. Furthermore, by determining individual behaviours, chromosomes collectively shape the comfort level that the microhabitats currently provide. The environment retroactively exerts selection pressure on the flibs: the genetic algorithm mimics Darwinian evolution by evaluating the fitness function, random mutation and selective reproduction. The dynamic equilibrium observed in previous El Farol bar implementations has been replicated under different threshold levels, producing more permissive or more restrictive environmental conditions. Even populations of the simplest flibs with only one internal state can approach equilibrium. Increasing the number of states increases the efficiency of the algorithm in resource utilisation by promoting behavioural diversification through increasing genomic richness. Observation of the Gini index shows that resources are shared more equitably when the number of internal states increases: this is an emergent property that is not directly targeted by selection. Conversely, a narrower potential in genetic variability seems to lead a population to the formation of subgroups having unequal access to resources. Further studies are needed to clarify whether the system spontaneously develops a tendency towards low or high genetic diversity.

POSTER

Eligible for Best Poster Prize

# Multiple distinct opsins in *Octopus vulgaris*

Mariangela Norcia[1], Stefania Galdiero[2], Maria Luisa Chiusano[3], Gianluca Polese[1,4], Emanuela Cirillo[1,4],
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Coleoid cephalopods, including octopuses, squids, and cuttlefish, have developed an advanced visual system crucial for their survival based on complex mechanisms of light detection. This system is dominated by photoreceptors located in the eyes, however, besides retinal photoreceptors, cephalopods also possess extraocular photoreceptors located outside the central nervous system, specialized in detecting light characteristics such as intensity and duration. In *Octopus vulgaris*, recent studies have characterized and localized the rhodopsin kinase GRK1 in the suckers, skin, and retina, highlighting its role as an extraocular photoreceptor. In this scenario, we expanded our focus on rhodopsin and retinochrome, which are fundamental photopigments to the visual process in these animals. Here we consider the gene expression not only in suckers, skin, and retina but also in the Central Nervous System lobes dedicated to visual transduction: the optic lobe. We analyzed the gene expression level of three critical genes involved in light detection (GRK1, rhodopsin, and retinochrome) using Real-time PCR, and mRNA transcripts in four tissues and we localized them through whole-mount in situ hybridization in the optic lobe of O. vulgaris. Furthermore, we constructed a phylogenetic tree to compare these photoreceptors across different species and generated 3D models of the proteins to understand their structures and functions. Understanding the mechanisms of light perception and the cascade of molecular signals triggered by light could provide crucial insights into the evolution of the visual system in cephalopods, which is not restricted to classical organs but is a diffuse visual system. The study of new photoreceptors, their evolution and the comparison of light perception mechanisms across different cephalopod species, occupying different ecological niches, could further enrich our understanding of this fascinating system in the light of species behavioral differences.

# Phylogeography and conservation genomics

INVITED LECTURE

## Capitalizing on genetics and genomics to inform conservation in a biodiversity hotspot

Angelica Crottini

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Madagascar is an ideal place to study the effects of human impacts due to its exceptional levels of diversity and endemicity, coupled with ramping levels of deforestation, which led to the loss of more than the 90% of its original forest cover. With not much left to extend formal protection, Madagascar qualifies as a great case study to identify challenges and opportunities to optimize the effective conservation of its remining native biota. For example, intraspecific genetic diversity is a crucial component of biodiversity. However, there is a gap in understanding the spatial patterns of genetic diversity in Malagasy amphibians and reptiles. A notable challenge to achieve this understanding is determined by the inconsistent storage of metadata in genetic repositories. We developed an automated workflow to extract data and characterize these patterns of genetic diversity to achieve an understanding of how well Protected Areas represent them. With more than 415 described species of amphibians (and another 200 candidate species waiting to be described), all of which endemic to Madagascar and often microendemic to minute areas, Malagasy amphibians represent a unique conservation opportunity. I provide an overview on how genetics and, more recently, genomics is contributing to inform conservation and foster public awareness in this island of wonders.

TALK

# Population History Predicts Genetic Load from both Structural Variants and SNPs Across Distinct Ptarmigan (Lagopus spp.) Lineages

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While the importance of specific demographic histories in shaping patterns of genetic load conferred by deleterious single nucleotide polymorphisms (SNPs) has received considerable attention in the recent past, few studies have investigated the corresponding fitness consequences of structural variation in distinct evolutionary lineages. We performed heuristic-based filtering and rapid automated curation of short-read-discovered SV callsets from 99 re-sequenced individuals across two ptarmigan (Lagopus) sister species. High-confidence SV callsets reveal that the relative proportion of deleterious structural variants is consistently greater in small effective population sizes, but that the relative frequency of deleterious variants differs between populations having experienced temporary bottlenecks followed by partial recovery, in contrast to longer-term declines in Ne. Despite the Svalbard rock ptarmigan population exhibiting the lowest long-term coalescent Ne of all populations, it did not carry the highest masked and realised load for both SVs and SNPs. Crucially, these differences in genetic load may reflect differences in the type of bottleneck: the Svalbard population has likely experienced an initial founder event during post-glacial colonisation of the islands, while the Pyrenean rock ptarmigan population likely experienced a longer-term gradual decline exacerbated by recent inbreeding. Furthermore, similar to SNPs, ratios of non-neutral SVs to synonymous polymorphisms are higher for historically small versus large populations, showing that deleterious SVs are subject to loss and fixation by genetic drift and relaxed purifying selection. Realised and masked genetic load therefore reflect more recent differences in post-glacial demography and inbreeding. This underscores the importance of understanding nuances in distinct population histories when interpreting the potential effects of small effective population size on genetic load from both SNPs and SVs.

TALK

# From Wild to Tamed and Back: demographic reconstruction of Sardinian sheep from whole-genome sequences

Viviana Floridia[1], Dehasque Marianne[2], Pedro Morell Miranda[3], Paolo Mereu[2], Monica Pirastru[3], Nicolò Columbano[4], Salvatore Naitana[4], Giovanni Giuseppe Leoni[3], Torsten Günther[2], Mario Barbato[1]

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Currently, three sympatric sheep (*Ovis aries*) lineages are found on Sardinia: the Sardinian mouflon, thought to represent the feral remnants of the earliest domesticated sheep, the Pecora Nera di Arbus (hereafter Nera), a local breed showing ancestral features, and the Sarda sheep, strongly selected for milk production. Despite their cultural and agricultural significance, the evolutionary history of these three sheep lineages is still unclear. To unravel the evolutionary origins of the Sardinian sheep, we generated whole-genome sequencing data of 19 Sardinian mouflons, six Sarda and nine Nera sheep, and co-analyzed them with a dataset of over 30 wild and domesticated sheep lineages. We explored the population structure, built a phylogenetic tree, and investigated the ancestry components. Results confirmed production-driven introgression in Sarda and suggested a first translocation of the Sardinian mouflon lineage followed by a later introduction of the domestic lineage originating Sarda and Nera sheep. Finally, we performed demographic reconstruction. We confirmed the time of emergence of the early domestic lineage at ~12,000 YA, from which the most modern domesticated sheep split around ~6,700 YA. Finally, we dated the split of Sarda sourcing from the Nera lineage around ~4,800 YA. Further analysis will reveal potential gene flow between the three lineages and allow the detection of genes involved in local adaptation and agronomic traits.

TALK

# The Geological-Ecological Molecular Clock Calibration: when ecological vicariance aids tree dating. A treefrog case study

Michael J. Jowers[1,2,3], Jesús Muñoz Pajares[1], John Weber[4], Jeanette Arkle[5], Salvador Arenas-Castro[6], Simon Y. W. Ho[7], Gilson A. Rivas[8], John C. Murphy[9], Amaël Borzée[10], Alexander R. Pyron[11], Marcos Perez-Losada[11], Mayke De Freitas[8], Roger Downie J.[12], Gregorio Moreno-Rueda[3,13], Giovanni Forcina[14], Santiago Sánchez-Ramírez[15]

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Assessing the timing of species divergence is crucial to understanding the underlying evolutionary processes. Nevertheless, molecular clock estimates often rely on calibrations based on external information, which might be either missing or partially available, such as fossil evidence or geological events. In the rare event that ecological information is also available, it can be incorporated to improve the accuracy and precision of the geology-based clock calibrations. In this study, we rely on the mutualistic relationship of a Neotropical monotypic hylid frog - *Phytotriades auratus* - and its bromeliad host - *Glomeropitcairnia erectiflora* - living in two isolated mountain ranges, as part of an island-continental split (Paria Peninsula, Venezuela - and island of Trinidad, Republic of Trinidad and Tobago) to date a vicariant event and calibrate a large hylid phylogeny through sequence data for the hylid *P. auratus* on each side of the intervening marine barrier. We also used ecological niche modeling to reconstruct the distribution of the frog and its host over the last 3.3 million years. We found that when using the Geological-Ecological Molecular Calibration (GEMC), the Arboranae divergence times were older than those inferred using fossil calibrations alone while age estimates for subfamilies and genera were, on average, 2 Ma and 1.2 Ma older, respectively. In conclusion, our study shows how biogeographic calibrations relying on geology and ecological data greatly reduces the uncertainty in date estimates for the shallower nodes in the phylogeny of the Arboranae. We also produced robust estimates of substitution rates that can be used in future dating studies of amphibians.

TALK

# Disentangling the Genetic Variation in the Hybridization Process Among Marmoset Monkeys of the Genus Callithrix (Primates: Platyrrhini) in Rio de Janeiro, Brazil

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Hybridization is recognized as a significant evolutionary process, especially in the context of conservation challenges. In Rio de Janeiro, Brazil, the endangered buffy-tufted-ear marmoset (*Callithrix aurita*) faces genetic introgression from anthropogenically introduced *Callithrix jacchus* and *C. penicillata*. These species produce fertile hybrids, complicating conservation efforts due to elimination of independent evolutionary trajectories (i.e., extinction by hybridization). To investigate the genetic diversity and hybridization dynamics, we analyzed genomic data (ddRADseq) from *C. jacchus*, *C. penicillata*, *C. aurita*, and their hybrids from various localities. Genomic DNA was extracted and libraries were prepared using SbfI-HF and MspI restriction enzymes. Sequencing was performed on an Illumina HiSeq platform, followed by data processing through demultiplexing, quality filtering, alignment to the *C. jacchus* reference genome, and SNP calling using the Stacks software pipeline. We then used R libraries and Structure software to analyze admixture and population structure. Our findings reveal extensive hybridization, with *C. jacchus* being the predominant parental species in hybrids. Notably, hybridization with *C. aurita* was confirmed in its native mountainous regions, identifying these areas as conservation priorities. The genetic data suggest a hybrid swarm resulting from multiple generations of hybridization and introductions, rather than first-generation hybrids. Extensive admixture was observed, primarily confined to contact zones, and the genetic variation was higher in hybrid populations relative to parental populations. These results highlight the complex interplay of genome architecture, local selection, and admixture in shaping the genetic landscape of hybrid marmoset populations, and underscore the need for targeted conservation efforts to preserve the unique genetic identity and evolutionary potential of *C. aurita* amidst the pressures of hybridization.

TALK

# Genome-wide diversity and population structure in the African buffalo (*Syncerus caffer*) in Mozambique

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Over recent centuries, the African buffalo has declined due to range contraction and human activities. Particularly, in Mozambique, historically home to large buffalo herds, the combined effects of civil war and poaching have created a critical situation. Information on genetic diversity of this species in Mozambique is scarce despite being necessary to plan conservation actions. Here, we analysed 72 buffaloes from six conservation areas to assess the genetic diversity of *S. caffer*. DNA extracted from blood was used to amplify the mtDNA control region (d-loop) and produce a genome-wide SNP panel. The d-loop was used to investigate phylogeographic patterns and historical demography, while SNP markers to infer population structure, admixture, recent demographic dynamics, gene flow, and genome-wide inbreeding. Mitochondrial analyses indicated a historically large and potentially panmictic population in Mozambique with demographic expansion until about 200K ya. However, SNP analysis revealed population differentiation between southern and central Mozambique. An unexpected genotype was found in Gorongosa NP, sharing a common ancestry with southern populations. All studied populations showed signs of genetic diversity erosion, high levels of inbreeding, and several genome regions in homozygosity. Our results suggest a past connection between southern and central Mozambique, with current population structure likely reflecting recent anthropogenic events. High levels of homozygosity indicate extensive inbreeding over recent decades, increasing the frequency of recessive deleterious alleles. Enhancing the resilience of *S. caffer* in Mozambique is crucial for ecosystem integrity. The most effective approach seems to involve facilitating translocations and re-establishing connectivity between isolated herds. However, it is essential to assess intraspecific genetic diversity when selecting suitable source populations for interventions to improve population viability.

TALK

# Infer the past to understand the future: past demographic history and environmental niche in tortoises and terrapins

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The recurrent climatic fluctuations of the Quaternary resulted in subsequent glacial and interglacial periods characterized by repeated changes in environmental conditions and resource availability. Several biotopes disappeared entirely or changed considerably leading to either extinction, survival in glacial refugia or adaptation to new habitats. Those environmental and distribution shifts likely had genetic consequences, with rapid demographic changes causing loss in genetic diversity. Among vertebrates, turtles are one of the most endangered group due to illegal trade, habitat loss, pollution, and climate change. Their vulnerability is worsened by peculiar life history traits such as delayed maturity, extended reproductive life and longevity. Current climatic alterations are already affecting several of their physiological and phenological characteristics. Similar modifications likely have occurred in the past, resulting in alterations to both effective population size and population structure. To investigate how climate and habitat modification influenced genetic diversity and past demographic history in Testudinidae, we inferred historical effective population size (Ne) using the Pairwise Sequentially Markovian Coalescent (PSMC) method and current Heterozygosity (He) using whole-genome sequencing data from 22 species of turtles. We then coupled effective population size with estimated suitable habitat during the Marine Isotope Stage 19, the last interglacial period, the last glacial maximum and the early Holocene. We analysed the correlation between He, Ne, area of available habitat and extinction risk. All species experienced a drastic decrease in Ne at the start of the last glacial period, statistically correlated to the decrease in temperature. Instead, no correlation was found between area of available habitat, effective population size, extinction risk and heterozygosity.

TALK

# Disentangling the global phylogeography of *Caretta caretta* by whole mitochondrial DNA analysis

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The loggerhead sea turtle (*Caretta caretta*) is a globally distributed, vulnerable species whose evolutionary history and colonization dynamics are still under debate, with some open questions. Which mitochondrial DNA (mtDNA) haplogroup first settled in the Atlantic, and how did the colonization of the Mediterranean occur? Why is only one mtDNA haplogroup found in nesting Eastern Mediterranean populations, out of two currently present in the Atlantic? We address these questions by analysing whole mtDNA sequences, obtained by widespread sampling. Our dataset comprises 27 complete mitogenomes (21 assembled in this study and 6 downloaded from public databases), from Greece, Turkey, Libya, Calabria, Florida, Mexico and Cape Verde. Through maximum likelihood and Bayesian phylogenetic reconstruction, we verified that the tree topology based on complete mitogenome sequences, and the consensus tree built using individual gene trees are different from the topology obtained by the analysis of mtDNA control region only. Our well-supported phylogenetic trees indicate that the exclusively Atlantic haplogroup separated from the other haplogroups and colonized the Atlantic Ocean during the closure of the Panama Isthmus (13-2.5 million years ago). The colonization of the Mediterranean Sea probably occurred later in the Pleistocene via a South African route by loggerheads coming from the Indo-Pacific. By studying non-synonymous mutations in coding regions and modelling protein structures, we also investigated the different selective pressures between geographically separated haplogroups of *C. caretta* and suggested an effect of temperature on mitochondrial DNA genetic differentiation and current mtDNA haplogroups distribution. Our study shows the potential of whole mitogenome analysis for a better understanding of the evolutionary history of sea turtles compared to classical analysis of single mitochondrial DNA sequences.

TALK

# *Esox flaviae* population genomics revealed entangled origin and differentiation of the Italian populations

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In 2011, *Esox flaviae*, commonly known as the Southern pike, was identified as a distinct species from the Northern pike (*Esox lucius*). This species is classified as endangered on the IUCN Red List because of habitat degradation and the threat of hybridization with alien E. lucius, which was introduced for angling purposes. This work aims to use a large set of SNPs to infer population structure and evolutionary dynamics to help define better management plans. Little has been done so far to study this species, but understanding the population dynamics is crucial to protect *E. flaviae* from local extinction, loss of genetic diversity, and loss of local adaptation. In this study, the genomes of 62 pikes were sequenced, including 50 *E. flaviae* from six locations (Alto-Adige, Trentino, Garda, Po Adda, Trasimeno) and 12 *E. lucius* from two locations out of Italy. The SNPs were examined to detect genome-wide hybridisation between species and admixture among populations. From the population structure analysis, we highlight the highly admixed state of the Po population, whereas the other population seems to be isolated. The sliding window phylogeny approach employed indicates variable relationships among populations through the chromosomes suggesting admixture. To distinguish between admixture and incomplete lineage sorting, we calculate the D-statistic tool that points to admixture events not evident in the initial structure and phylogenetic analysis between Adda and Trasimeno. Such mixing, likely caused by human-mediated translocation for angling, complicates conservation efforts. Preserving the adaptive potential of a species or population is crucial for its ability to adapt to changing environments. In this framework, this study lays the foundation for determining Conservation Units (CUs) and Evolutionary Significant Units (ESU) essential for developing informed management plans to protect this species.

POSTER

Eligible for Best Poster Prize

# Revealing the population structuring of the stingless bee *Tetragonisca angustula* (Apidae: Meliponini) in Peruvian Amazon

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The stingless bee *Tetragonisca angustula* (Apidae: Meliponini) not only represents a fundamental component of the pollination system of the Amazonian tropical forests (Francisco et al. 2016, Insect Science 24: 877–890), but also one of the most reared species in traditional meliponiculture (Quezada-EuÃ¡n et al. 2018, Sociobiology 65: 534-557). Knowledge on the intraspecific genetic diversity of this stingless bee species may contribute to its conservation and development of meliponiculture (Marconi et al. 2022, Diversity 14: 632). We evaluated the genetic variability and geographic structure of 47 specimens of T. angustula collected along a transect spanning 1630 km in 4 regional departments of Peru during June-September 2021/2023. First, we identified through mtDNA molecular markers (COI, Cytb) 3 main haplogroups partially coherent with the geographic origin of the analyzed samples (south vs. central+north). We observed overall a p-diversity of 0.008 (COI) and 0.019 (Cytb), with the southernmost populations showing the lowest mtDNA diversity. The 13 microsatellite loci grouped *T. angustula* peruvian populations in two main genetic clusters, one including all southernmost individuals ("south"), whereas the other those from central/northern areas ("north/central"), with the exception of 4 admixed individuals. The "south" group showed a He = 0.77, and 14 private alleles with respect to the "north/central" cluster (He = 0.69 and 55 private alleles). The peculiar genetic isolation of the southern populations (i.e. the "south" cluster) of *T. angustula* could be ascribed to their localization in the Biogeographic Province of Ucayali (Morrone 2017, Neotropical biogeography: regionalization and evolution. Crc Press), known to be historically separated from the northernmost provinces by ecological (e.g. a more humid climate) and physical (e.g. offshoots of the Vilcabamba mountain system) barriers. Further data on genetics, pollen and habitat suitability are in progress to better understand the ecological requirement for this pollinating species and its role in meliponiculture.

POSTER

Eligible for Best Poster Prize

# Environmental DNA (eDNA) as a tool to evaluate the effectiveness of Natura 2000 network in conserving Central Italy freshwater-associated biodiversity

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In recent decades, a massive loss of biodiversity has affected terrestrial, freshwater and marine ecosystems. Population trend data show that the rates of decline in freshwater species are consistently higher than those on land. This is an alarming trend, since freshwater habitats are among the most diverse ecosystems on the planet, hosting almost 9.5% of all known animal species. The designation of protected areas is a key conservation strategy to halt biodiversity decline. The largest coordinated network of protected areas in Europe is the Natura 2000 network, which preserves among the most valuable and threatened species and habitats, both on land and at sea. The effectiveness of terrestrial protected areas, however, has been questioned for the freshwater habitats they encompass, and many studies showed the need to improve monitoring programmes. In this study, we used an integrated approach to assess the effectiveness of protection status for preserving watercourses and their associated biodiversity. We characterised six pairs of streams in the central Apennines (Tuscany, Italy) within the same hydrological catchment and under the same climatic conditions. Each pair included a stream placed inside and one selected outside of Natura 2000 areas. We measured environmental parameters (e.g., sediment granulometry, canopy coverage, water depth) and water quality parameters (e.g., minimum and maximum temperatures, dissolved oxygen, pH, major and minor ions concentrations) to evaluate the quality of the sites. We then assessed species richness and diversity using an environmental DNA (eDNA) multi-marker metabarcoding approach. The eDNA results allow to detect vertebrate and invertebrate species, either living in or occasionally visiting the watercourses, and to reconstruct trophic networks. Data on community richness and stream quality parameters were analysed using univariate and multivariate statistical analyses to test the effectiveness of freshwater ecosystem protection.

POSTER

# Genomic Insights into the Conservation of the Endangered Hermann's Tortoise (*Testudo hermanni*)

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The Hermann's tortoise (*Testudo hermanni*) is an endangered land tortoise distributed in disjoint populations across Mediterranean Europe. Habitat reduction, intensive agricultural practices and forest fires are major causes of decline in different areas. Intense harvesting for the pet trade and release of non-native individuals into local populations represent additional threats. Our previous genetic studies based on STR and ddRADseq markers were able to clearly distinguish two subspecies (the eastern *T. h. boettgeri* and the western *T. h. hermanni*) and to identify some major geographical groups, enabling the development of practical genetic tools for geographic assignment. More recently, we generated a high-quality assembly and annotation of the *T. hermanni* genome using a combination of PacBio HiFi and Oxford Nanopore Technologies data. The assembled genome size was 2.26 Gb with a contig N50 of 58.76â€‰Mb. The genome included 22,017 protein-coding genes and repetitive elements constituted 45.5% of the assembled genome. The genome assembly and the set of annotated genes yielded 97% and 95% completeness scores, respectively, when compared with the BUSCO Sauropsida dataset. In addition, we re-sequenced whole genomes at ~20x coverage of six individuals, three for each subspecies. We confirmed a clear distinction among the two subspecies, and the major geographical groups. Demography inferences revealed that the two subspecies diverged ~400k years ago during a period of warming temperatures, ice retreat and rising sea levels. Both subspecies experienced a marked bottleneck during the last glacial maxima (LGM), followed by a slight demographic recovery, especially for the eastern subspecies. Overall, we found lower genomic diversity in the western subspecies, comparable with other threatened reptiles worldwide. The highest inbreeding levels were found in small and isolated populations from France (western subspecies) and Bosco Mesola (eastern subspecies). We are further investigating genomic landscapes, focusing on among-subspecies summary statistics of diversity and divergence. Thus far, we have found an elevated absolute divergence (dxy) in genes involved in immune responses and olfactory receptor activity. These results will allow a better understanding of the effective risks of translocation and hybridization in *T. hermanni* and help devising management plans based on functional genomic regions.

POSTER

# EasyAmpR: genotyping of multilocus amplicon sequences in R

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The massive sequencing of amplicon libraries allows the parallel sequencing of dozens to thousands of loci for many samples in a single experiment. The biases, sequencing quality, and the large amount of data impose some challenges in the genotyping of samples from this type of genetic data. EasyAmpR is the first R package designed to automate the genotyping of diploid individuals from amplicon library sequences obtained through massive sequencing, such as Illumina MiSeq or NovaSeq. It proposes a workflow that starts with the demultiplexing of loci, variant determination with dada2, and genotyping from the variants. Additionally, it includes several useful functions for format conversion and exporting results in the form of tables and FASTA files. We successfully tested EasyAmpR in multiplex libraries of 30 loci for a population genetics study of a Southeast Asian rodent species, comparing the sequences generated by EasyAmpR with published data for the same species.

POSTER

Eligible for Best Poster Prize

# Fighting extinction using genomic tools: The First Reference Genome of the Italian brook lamprey (*Lampetra zanandreai*)

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Historically, lampreys (Petromyzontidae) have held a central role in the quest to understand the origin of the vertebrate body. Their morphological characters position them as a sister group to the jawed vertebrates (Gnathostomata), making them the most ancient group of living vertebrates. Their ancient lineages and relatively unchanged body structures over millions of years make them prime targets of the study of evolutionary biology and comparative genomics. Despite their evolutionary significance, lamprey populations in Italy face extinction threats from habitat degradation, water pollution, dam construction, and invasive species. The decline of wild animal populations as a result of anthropogenic influences poses a substantial threat to the long-term survival of species. In fact, rapid population declines can limit genetic diversity and adaptability to changing environments. Northern Italy, habitat of the Italian Brook Lamprey (*Lampetra zanandreai*), is heavily impacted by anthropogenic pressures, leading to native habitat destruction and species displacement. This highlights the urgent need for effective conservation programs. Nevertheless, to enable the analysis of population and conservation genomics, it is essential to generate reference genomes for species under investigation. In light of this, we present the assembly and annotation of the first reference genome of the Italian brook lamprey. Whole genome and transcriptome sequencing was done with Oxford Nanopore Technology, DNB Sequencing and Hi-C sequencing to obtain long and short reads, and the three-dimensional structure of the genome, enabling the correct scaffolding of assembled contigs into chromosomes. This reference genome is fundamental for lamprey conservation efforts in Italy, as it provides a solid genetic basis to understand their biology, monitor their genetic diversity and develop effective conservation strategies.

POSTER

Eligible for Best Poster Prize

# World-wide population structure of the loggerhead sea turtle revealed by whole genome sequencing

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The biodiversity crisis demands urgent conservation actions to halt and reverse the situation. In this context, conservation genomics can assist biodiversity conservation and management with reference genomes being key centrepieces in such analyses. However, no single genome can represent the diversity of a species, as structural and sequence polymorphisms may differentially drive the future conservation actions of populations. We aimed to go beyond the reference genome of the loggerhead turtle by adding nuclear and mitochondrial genomes from individuals sampled worldwide. We performed whole genome sequencing (> 30 Gb output) on 16 individuals from four different Regional Management Units (RMU): Pacific (Mexico: 2), western Atlantic (Mexico: 2; Florida: 2), eastern Atlantic (Boavista: 5) and Mediterranean (Libya: 1; Greece: 2; Turkey: 2). We genotyped all samples by mapping their reads against the reference genome (GCA_023653815.1) to produce a set of nuclear SNPs across all individuals. We performed multidimensional scaling to see how the individuals from different RMUs cluster and we calculated the genetic distances, measured as Fst, to compare if the two types of analyses show the same pattern. We performed pairwise Fst comparisons by chromosome and an analysis of Runs of Homozygosity (ROHs) was done to show the degree of inbreeding across RMUs. In summary, we think that combining the reference genome with whole genome sequencing data worldwide can provide novel insights into marine turtle conservation genomics. Future efforts to increase sample size and to incorporate more RMUs are advisable to expand our comprehension of marine turtle genomics for conservation.

POSTER

# Shotgun metagenomic sequencing for conservation genomics: rock ptarmigan as a case study

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The rock ptarmigan (*Lagopus muta*) inhabits alpine and arctic tundra throughout the northern hemisphere with its current distribution mainly determined by the last glacial maximum. In southern Europe this species is only found in fragmented populations on the main mountain ranges, where numbers are decreasing due to habitat loss, degradation and fragmentation, climate warming and human disturbance. This decline could be mitigated through conservation measures, but these require fundamental information on the evolutionary ecology, distribution and genetic diversity. Such data is difficult to collect in a species which is particularly elusive and living in extreme environments. Thanks to the technological and computational advances of recent years, understanding of species' biology has been greatly enhanced by analyzing fecal samples, collected non-invasively in the field, using shotgun metagenomics sequencing. Although this approach is still poorly tested in wild species, ongoing studies highlight its potential for conservation and biomonitoring that should be further explored. In the present study, this approach was applied to 30 rock ptarmigan from three different areas of the Paneveggio-Pale di San Martino Natural Park, characterized by contrasting rocky substrates. The shotgun metagenomic sequencing method does not require the enrichment or targeted amplification of specific molecular markers, thus allowing both qualitative and quantitative evaluation of data on different aspects related to the species. In particular, the ongoing analyses aimed to determine: i) the genomic relationships between individuals and population, ii) the fundamental resources for the species through the study of the diet, iii) the health status of individuals through the analysis of gut microbiota and parasites. First results confirmed the utility of shotgun metagenomic sequencing for conservation purposes and provides fundamental data for improving conservation strategies for this species.

POSTER

Eligible for Best Poster Prize

# Shedding the light on the evolutionary paths and modern diversity of wolves (*Canis lupus*)

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Over the past millennia, human activities have caused the wolf (*Canis lupus*) to undergo phases of expansion and contraction, resulting in a complex structure of populations and subspecies throughout the Holarctic region. The mitochondrial genetic diversity of wolves has typically been described using two current mitochondrial haplogroups, called W1 and W2, which indicate two main described clades. Additional haplogroups, now extinct, have also been identified. However, many haplotype relationships remain ambiguous. This study aims to understand the factors that have contributed to shaping the current population structure and to investigate why the Italian wolf population is uniquely represented only by haplogroup W2, while other populations have haplotypes belonging to both haplogroups. To achieve this goal, a database of over 800 mitogenome sequences (47 from this study) was constructed. This database includes modern, historic (<700 years ago), and ancient (>700 years ago) samples of both dogs and wolves. The sequences were aligned using the entire mitochondrial genome and were later used to perform coalescence analysis and Skyline reconstruction. Our results indicate a clear separation between the W1 and W2 clades that occurred around 50,000 years ago, and an earlier separation of modern wolves and dogs from three extinct wolf lineages around 80,000 years ago. Almost every node of the phylogenetic tree is supported by high posterior probability values. The Skyline reconstruction shows a continuous increase in population over time, followed by a decline in the last three millennia. The extensive dataset proved useful for reconstructing the evolutionary history of the wolf, revealing with unprecedented resolution the subdivision of different lineages. Future genomic studies based on nuclear DNA analysis may provide even greater insights than those obtained so far using mitochondrial DNA.

POSTER

# Genomic data uncover evolutionary histories of Galápagos iguanas

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Galápagos iguanas are a monophyletic group endemic to the Galápagos archipelago which comprises the marine iguana (*Amblyrhynchus cristatus*) and three species of land iguanas: *Conolophus subcristatus*, *C. pallidus* and *C. marthae*. The IUCN classifies *C. marthae* as Critically Endangered and the others as Vulnerable. Our work aims to elucidate the processes that led to the current distribution of Galápagos land iguanas across the archipelago. By reconstructing past demographic fluctuations and divergence times, we can clarify their evolutionary histories and colonization patterns, shedding light on species distributions. This is especially useful for species with unexplained geographic distributions, like *C. marthae*, or with debated species status, like *C. pallidus*. The lineage that gave rise to *C. marthae* split earlier than the other two congeneric species, yet it is restricted to a small area of one of the youngest islands of the archipelago, Isabela. In contrast, *C. subcristatus*, which diverged later, is widespread across many islands. *C. pallidus* is found exclusively on the small Island of Santa Fe. Our RAD-seq analyses indicate that the ancestors of Galápagos iguanas likely reached the archipelago ca. 10 Mya. In contrast with all previous reconstructions based on mtDNA, our data would also indicate that *C. marthae* diverged recently after the emergence of Isabela, still implying an allopatric speciation model. Our data, that reveal recent demographic trajectory of *C. marthae* in contrast to the syntopic *C. subcristatus* population, indicate also a competitive interaction possibly due to a second colonization of Isabela by *C. subcristatus* itself. We confirm the recent divergence of *C. pallidus* from *C. subcristatus* due to intense genetic drift in a historically small population. Our findings have significant implications for the conservation of Galápagos iguanas, emphasizing the need for target strategies.

POSTER

Eligible for Best Poster Prize

# Genetic variability, phylogeography and demographic history of the tomato leafminer *Tuta absoluta* (Meyrick) populations in Tunisia.

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The South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most serious invasive and destructive insect pests, causing extensive damages mainly to tomato *(Solanum lycopersicum* L.) crop. The aim of the present study was to investigate the genetic diversity of *T. absoluta* along a latitudinal gradient in Tunisia, to gain information about its demographic history and population structure. We amplified and analysed the cytochrome oxidase subunit I (COI) gene and the nuclear Internal Transcribed Spacer marker (ITS) for 96 *T. absoluta* specimens collected from ten localities. The results revealed a high genetic uniformity in *T. absoluta* populations both in Tunisia and worldwide, characterized by low haplotype and nucleotide diversity. This suggests that the Tunisia population may have undergone rapid demographic expansion following its recent colonization. Knowledge of genetic diversity and differentiation, as well as population structure, is essential for implementing control strategies against this pest.

POSTER

Eligible for Best Poster Prize

# Genomic insights into postglacial expansion dynamics of an Italian endemic amphibian: *Bombina pachypus*.

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Geographic range expansion affects population dynamics and consequently shapes biodiversity distribution. Moreover, dispersion and colonization of new habitats may lead to genetic diversification as populations face environmental changes. In this study, we investigate patterns of genomic variation as a result of postglacial expansion in the Italian Apennine yellow-bellied toad, *Bombina pachypus*. Like many other amphibians, *B. pachypus* has a large (10 Gb) and complex genome. We aim at assessing genetic diversity and genetic load by targeting 24 Mb of genomic regions including high-effect and low-effect genes, regulatory sequences and neutral intergenic areas. These regions will be selected by means of open chromatin sequencing (ATAC-seq) and mRNA sequencing. Custom probes will be designed to capture target sequences and employed to prepare individually-barcoded libraries for wild toads sampled across the species' range. We will estimate genetic diversity and gradients of genetic load along the route of expansion. Signatures of natural selection will be inferred by detecting positive selection in stable southern populations and by tracking shifts in fitness peaks at the expanding front of the range. As to date, we performed ATAC-seq analysis on two biological replicates to select regulatory target regions using the PEPATAC pipeline. Data were aligned to the *B. pachypus* reference genome, repetitive regions were masked and consensus sequences of open chromatin were identified and annotated. The next step involves selecting regulatory regions to include in the probe design process.

POSTER

# The end of the American dream; how the barn swallow (*Hirundo rustica*) returned to Europe

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The barn swallow (*Hirundo rustica*) is one of the most iconic and fascinating migratory birds since ancient times, their philopatry and pair bonding have been the symbol of travellers and lovers throughout history. The barn swallow subspecies complex has been the main focus of the latest papers, with the main focus being on Eurasian and Levant subspecies (*H.r. rustica* and *H.r. transitiva*). The remaining subspecies have either not been studied or few mitogenome sequences have been published. In this work we obtained complete mitogenomes of 8 *H.r. savignii*, 1 *H.r. gutturalis*, 8 *H.r. erythrogaster* and 8 never before published *H.r. tytleri*. In addition, we included 13 hybrid tytleri-gutturalis and 13 rustica-tytleri. We date the swallow entry in the Americas ~146 kya and confirm the previously hypothesised back migration into Russia ~51-79 kya. Moreover, we found two major haplogroups of *H.r. tytleri* deriving from a major new haplogroup of *H.r. erythrogaster*. Hybrid zones revealed only tytleri mitogenomes unlike nuclear studies, indicating signs of sexual selection. Finally demographic analysis with the addition of Russian individuals shows correlation to climactic events, with the LGM and YD being the main actors on the species dispersion back into mainland Eurasia. The addition of this subspecies also pushes back the molecular dating for the Swallow Ancestral mitogenome (HrAM) to 389 kya, ~100 ky more than what we previously thought.

POSTER

# Conserving threatened saproxylic beetles in Italy: a genomic approach

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A genomic approach in conservation biology may provide valuable insights to the understanding of processes contributing to the adaptation of endangered species and to assess the viability of populations that are currently restricted to fragmented habitat patches. *Rosalia alpina* (Coleoptera: Cerambycidae) and *Osmoderma eremita* (Coleoptera: Cetonidae) are saproxylic species, thus strictly associated with dead wood, depending on dying and decaying trees in mature forests. Both taxa suffer of habitat loss in Europe and, hence, are protected and included in the IUCN Red List of Threatened Species. In Italy, *Rosalia alpina* counts a not yet well-defined number of evolutionary significant units, whereas two endemic species of hermit beetle, *O. cristinae* and *O. italicum* - phylogenetically related to the more largely distributed *O. eremita* - were described in Sicily and Southern Italy, respectively. In the frame of the activities promoted by the National Biodiversity Future Center, we aimed to: 1) unravel the phylogeographic pattern of *R. alpina* in Italy employing nWGS and dd-RADSeq to better define potential evolutionary significant conservation units and reveal the degree of genetic erosion over time; 2) conduct a comparative genomic study on Osmoderma spp. in Italy to gain a better overview on the genomic differentiation among *O. eremita* vs. *O. cristinae* and *O. italicum* and to possibly identify expanded/contracted gene families contributing to local adaptations. The obtained genomic data will be also interpreted in light of the effects of the adopted protection measures and landscape variables in collecting areas, allowing for the identification of those habitat fragmentation drivers informative for future conservation actions of old-growth forests.

POSTER

Eligible for Best Poster Prize

# An initial investigation into the genetic diversity, phylogeographic relationships, and population structure of the Olive Psyllid, *Euphyllura olivina*, in Tunisia

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The olive psyllid, *Euphyllura olivina* Costa (Hemiptera, Psyllidae), causes considerable damage in the olive groves of the Mediterranean area, resulting in significant loss in olive oil production. The present work aims to gain a first overview on the genetic diversity of this species in Tunisia by analysing patterns of genetic variability in the mitochondrial cytochrome oxidase subunit 1 (COI) and the nuclear Internal Transcribed Spacer marker (ITS). Our results reveal low genetic differentiation among the studied populations, with low nucleotide and haplotype diversity. These outcomes are associated with frequent rare variants and high gene flow exchange, suggesting recent colonization followed by population expansion across the invaded range. We cannot exclude the possibility that the low genetic diversity results in part from a reduction in the population size associated with poor performance under the current climatic conditions. Future investigations could benefit from the inclusion of additional genetic markers, including microsatellites, or population genomic approaches, to more accurately trace the demographic history and genetic differentiation of this pest insect.

POSTER

Eligible for Best Poster Prize

# MIPs-Based genomic insights into Pike hybridization and diversity

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Understanding contemporary potential hybridization between endangered and invasive species is crucial for evolutionary biology and conservation efforts. This study leverages MIPs (Multi-locus Intron Polymorphisms), an innovative category of nuclear markers successfully applied for species identification in Teleosts, to assess their efficacy in detecting hybridization compared to traditional microsatellite markers, using pike species as a case study. The Southern pike (*Esox flaviae*), native to the Italian peninsula, is facing a drastic decline due to habitat degradation and overexploitation, compounded by the invasive spread of the Northern pike (*Esox lucius*). Genetic studies reveal distinct evolutionary lineages, indicating a relatively recent divergence between these two species. However, the occurrence of Northern pike traits within Southern pike populations suggests potential historical interspecies interactions and recent hybridisation events, likely facilitated by human activities such as reintroductions and translocations. To tackle this issue, we used the best 47 functioning loci in pike species from the original panel used for Teleost identification and we analysed the genomic DNA of 188 pike samples, sourced from wild sites and aquaculture facilities, through library preparation and MiSeq Illumina sequencing. MIPs results were also compared with previous findings obtained from the genotyping of 16 microsatellite loci. Our results revealed that MIPs effectively differentiate between the two pike species and confirmed the presence of interspecific hybrids in various pike populations with comparable efficiency of microsatellites. Furthermore, sequence information obtained suggests the potential of developing diagnostic PCR-based markers for species and hybrid detection. These findings highlight the MIPs power for vast applications in conservation genomic studies.

POSTER

# TE abundance and dynamics in the large genome of *Bombina pachypus*

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Genome sizes exhibit significant variation (up to 200,000-fold) among eukaryotes. Genome size variation is mainly influenced by changes in the non-coding regions of the genome, through variations in the proportion of repetitive and mobile elements (TEs), and the amount and length of introns. Such an extreme variability in genome size evolution could be a trait under selection due to its direct phenotypic effect on organismal fitness (adaptive hypothesis) or just a consequence of relaxed selection and increased genetic drift in small populations (non-adaptive hp). Yet, this still remains a subject of controversial debate. By investigating the genomic distribution of TEs and their ancient and recent expansion dynamics, here, we study the mechanisms underlying genome gigantism in the Italian endemic Apennine yellow-bellied toad, *Bombina pachypus*, featuring one of the largest genomes among anurans (10 Gb). After assembling a chromosome-level genome, we first describe the abundance, diversity and genome localisation of the different TE families in *B. pachypus* in comparison with its closest species, *B. bombina*, and eight other anuran species for a range of genome sizes from 1 to 10 Gb. Then, we use TEs expression data for *B. pachypus* and de novo sequenced genomes from populations of this species with different demographic history to investigate the most recent dynamics of TE activity. Our results highlight the predominant role of TEs amplification in driving genome size expansion in Anurans, as evidenced by a significant positive correlation between genome size and TE copy numbers across species, showing no substantial alterations in TE family diversity, but rather an increase in the copy number of TEs in multiple families. We also observed increased TEs abundance in the population characterised by increased genetic drift, suggesting TEs expansion is a non-adaptive process. Why only a few orders of animals runaway to genome gigantism remains an open question.

INVITED LECTURE

# The roles of hybridisation and chromosomal rearrangements in rapid adaptive radiation

Joana Isabel Meier

*Wellcome Sanger Institute, UK*

TALK

# Is domestic introgression affecting the Italian wolf behavior and potential interactions with human-dominated environments?

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The Italian wolf (*Canis lupus italicus*), after recovering from the brink of extinction, has experienced anthropogenic hybridization with domestic dogs (*C. l. familiaris*) in the last three decades. Domestic introgression affects several morphological traits of the Italian wolf, but we lack understanding of whether it could also influence wolf behavioral and physiological traits. This phenomenon might concern human society if wolves become more confident and potentially problematic due to introgression. Therefore, we analyzed good-coverage whole-genomes (~15x) of 17 Italian wolves sampled in the core area of wolf distribution in Italy. After discriminating admixed and non-admixed wolves through admixture analyses, we carried out local ancestry inference, selection signatures and gene ontology analyses to understand whether adaptive and recent introgression of domestic variants could influence wolf interactions with anthropic environments. Additionally, we examined if 300 genes associated with social behavior in previous studies, and highly differentiated between wolves and dogs, show a different domestic ancestry than expected by chance within admixed wolves. We found 18 recurrent and recently introgressed regions, but none resulted in significant functional profiling. We also found 50 recurrent and non-recently introgressed regions, potentially exhibiting adaptive introgression signs, located on genes that may affect response to stimuli and regulation of different biological processes. Nevertheless, we did not find a domestic overrepresentation in the reference behavioral genes of admixed wolves. These findings highlight that, although recent introgression might not currently influence the Italian wolf behavior, adaptive introgression could have an impact. Our study underscores the importance of managing the wolf-dog hybridization phenomenon to preserve the evolutionary trajectories of the Italian wolf and foster harmonious coexistence with human populations.

TALK

# The evolutionary history of Italy from Mesolithic to the Middle Ages: lessons from ancient genomes

Rajiv Boscolo Agostini[1], Stefania Vai[2], Maria Teresa Vizzari[1], Silvia Perretti[1], Federico De Pizzol[1], Alissa Mittnik[3,4,5,6,7], Donata Luiselli[8], Olga Rickards[9], Luca Sineo[10], Nicoletta Volante[11], David Emil Reich[3,4,5,6,12,13], Silvia Ghirotto[1], David Caramelli[2]

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Italy presents a complex history, due to its strategic position at the center of the Mediterranean Sea and to the presence, since Prehistory, of different groups and cultures. This could be caused by different levels of cultural and biological contributions from outside the country both in prehistoric and historical times, as well as the presence of different environments, climates and resources that could have led to cultural and/or biological differentiation and even isolation. We employ ancient DNA to investigate these complex dynamics analyzing genomic variation through time and space. To explore the peopling of the country from the Paleolithic to the Middle Age we collected 1,816 ancient human remains and analyzed them through a combination of target enrichment of nuclear informative SNPs and whole genome sequencing. We sequenced 43 ancient samples from 16 Italian necropoleis, obtaining whole-genome sequences with mean depth ranging from 0.03x to 9.5x. These new genomes, together with a dataset of 292 published ancient whole-genomes, have been analyzed employing a genotype likelihood framework, in order to account for implicit uncertainty of low-coverage data. We investigated the distribution of genomic variability through time employing multivariate and genomic structure analyses. Our results show different levels of external contributions in our new Italian samples, highlighting that genetic contributions from populations of Iran and Pontic Steppe from Chalcolithic to the Iron Age played a key role in the history of Italy. Furthermore, the use of genotype likelihoods allowed us to obtain phenotypic predictions for our new Italian samples. These results have been used to design demographic scenarios to be explicitly tested through an Approximate Bayesian Computation (ABC) framework optimized for low-coverage data. This approach allows us to explore, at an unprecedented level, the colonization dynamics and the genealogical relationships of the Italian peninsula through time and space.

TALK

# Phenotypic Evolution in the Apennine Brown Bears: How Much Does Epigenetics Matter?

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The relict and highly endangered Apennine brown bear (*U. a. marsicanus*) features a marked phenotypic divergence from other European populations. Recent studies suggested that such divergence might have adaptive significance and was likely triggered by isolation and inbreeding. In addition, 3D geometric morphometrics analysis suggested that the loss of morphological integration, which is the tendency of traits to vary jointly due to genetic and epigenetic interactions during development, may have enhanced phenotypic evolvability of brown bear. To date, understanding the drivers of phenotypic changes remains a challenging task, and additional information is needed. Over the past two decades, epigenetics has emerged as a crucial field for understanding the mechanisms behind phenotypic plasticity and adaptation. Hence, we performed a genome-wide DNA methylation analysis to investigate the epigenomic profile of different brown bear populations and explore its possible significance in terms of phenotypic evolution and adaptation. Tissue samples (n=12) from three European populations (Apennine, Alps, and Scandinavian peninsula) were analyzed using Reduced Representation Bisulfite Sequencing to infer and compare genome-wide methylation patterns in blood and muscle across populations. Differential methylation was assessed at both site and regional levels (DMR). Our results revealed that beyond tissue-driven differences, the methylation patterns were significantly distinct across the populations, highlighting the possible role of local environmental conditions in influencing gene expression. According to gene set enrichment analysis, many genes close to DMR are involved in development, supporting previous hypotheses that changes in developmental pathways may have contributed to the emergence of phenotypic novelties with potential adaptive significance in the isolated Apennine brown bear population.

TALK

# Human-bear coexistence: genomic and behavioral consequences in a small Italian population

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The Apennine brown bear (*Ursus arctos marsicanus*) is a relic population surviving in central Italy at low numbers. Its prolonged isolation from the other European populations led to marked genetic differentiation as well as morphological and ecological divergence. The Apennine bear is generally described as less aggressive compared to other brown bear populations, such as the European brown bear (*U. a. arctos*) and the North American brown bear (*U. a. horribilis*). While strong genetic drift has certainly shaped the genetic diversity of this endemic population, an alternative hypothesis suggests that prolonged persecution and environmental sharing with humans may have influenced the evolution of the bear and favored tamer individuals. To test this hypothesis, we compared whole genome sequences of Apennine bears (N = 11) with bears from Slovakia (N = 9), Alaska (N = 6), and Canada (N = 9) as controls. Three complementary genome-scan methods were used to identify signatures of selection in the Apennine bear. We identified 564 candidate genes under selection in at least one analysis. Functional enrichment analysis revealed that the human phenotypes overfriendliness and social disinhibition were enriched (seven outlier genes, p=0.038), suggesting a signal regarding behavioral mechanisms. Manual screening of the candidate set identified thirteen additional genes known to influence the behavior or the neurological development in other species with tamer/domesticated forms. Interestingly, these 20 genes showed highly differentiated intronic SNPs between the Apennine bear and the control populations involved in splicing alterations. This finding could imply that differences in mRNA maturation play a role in determining Apennine-bear specific phenotype. Our findings suggest human-mediated selection for a tame behavior in the Apennine brown bear.

TALK

# Pompeii: exploring the genomics of a city frozen in time

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Buried under volcanic ash since the eruption of Mount Vesuvius in 79 CE, Pompeii certainly is a time capsule of Roman antiquity. Its unique set of well-preserved buildings and human remains has captured the hearts of millions since its first excavations in the 18th century, leading to its inclusion on the UNESCO World Heritage List. Even though the analysis of the victim's DNA has been included in anthropological studies since the mid-1990s, recent advances in genomics and bioarchaeology now allow a deeper exploration of this emblematic Roman city. For the first time, we were able to study the genomic variability of 12 Pompeian samples sequenced at the University of Florence, with an average sequencing coverage of 1x. Given the low coverage obtained, we relied on probabilistic methods of analysis that explicitly take into account measures of uncertainty in genotype calling. Our analysis highlights the presence of a heterogeneous population with a wide genetic diversity: the individuals from Pompeii appear to be genetically differentiated from Imperial populations in Italy, while being closer to populations from the Near East. These results are confirmed by the analyses of individual ancestries, which show the presence of ancestral components typical of European populations - such as Anatolian Early Farmers' ancestry, Iranian Neolithic and Steppe component - while showing the absence of Western Hunther Gatherer's ancestry. The wide genetic diversity observed is also reflected in a wide phenotypic variability. Previous archeological and genetic studies have already underlined the significant impact of immigration from the Near East in Italy during the Imperial Age. In light of our findings, this event might have been even more significant for the population of Pompeii. Our study, therefore, confirms the relevance of this city as a crucial hub for cultural and genetic exchange within the Roman Empire and expands our understanding of a city that for centuries marveled people with its enigmatic aura and mysterious allure.

TALK

# Dissecting the structural and regulatory genomic basis of hypertrophied lip adaptations in Neotropical cichlid fishes

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Cichlid fishes offer powerful models to understand the molecular mechanisms that generate biodiversity. In cichlids, the repeated evolution of novel traits provides a framework to study the genomic basis of adaptation. For instance, hypertrophied lips have convergently arisen in several adaptive radiations, including the Neotropical Midas cichlid species complex (Amphilophus spp.), where a functional trade-off in feeding behaviour between thick- and normal-lipped ecotypes is likely fueling divergence through disruptive selection. In this study, we combined analyses of QTL, pangenomic, transcriptomic and chromatin interactions to characterize the molecular genetic bases of the hypertrophied lip phenotype in the Midas cichlids. Our findings reveal that more loci than previously thought contribute to variation in this trophic trait, including two loci with large effect size. Additionally, we identified several genes that show differential expression (DE) between fishes with thick and normal lips and link this to associated DE in miRNAs. Moreover, pangenome reconstructions resolved several ecomorph-specific structural variants that co-localize with lip QTL intervals. Lastly, topological associating domain (TAD) analysis revealed altered TADs in those genomic regions harboring coincident structural variants and QTL. Collectively, our results suggest that variation in lip size - a possible driver of sympatric speciation - is due to a complex interplay of multiple genomic factors.

TALK

# Genomics of plant adaptation to the harsh Arctic-alpine environment

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Plants with an Arctic-alpine distribution are challenged by extreme temperatures, low water availability and short favorable seasons, and can be used as models to study adaptation to harsh environments. Further, these species experience habitat loss and fragmentation due to climate change, and studying their evolution and genomic diversity can reveal possible responses to a warming climate. In order to study genomics of adaptation to the extreme Arctic-alpine environment, we have assembled a large-scale collection of more than 1200 individuals of the Arctic-alpine plant *Arabis alpina* collected across Europe, and we have sequenced their whole genomes. Using population genomics inference, we reconstructed the ancient spread of this plant across Europe, dating back 0.5 to 1 Million years ago, which accumulated tremendous genetic divergence between regions and populations. Further, we detected a widespread decline of diversity within populations, and increasing rates of drift since the last glacial maximum (ca. 20 thousand years ago), likely driven by warming temperatures in the present interglacial. During its expansion*, A. alpina* occupied contrasting environments, from Mediterranean mountains with long, dry growing seasons, to high altitude and high latitude sites with short growing seasons. Across the range, we find signatures of selection associated with genes related to drought response, with climatic variables such as aridity indexes, and with large genomic structural variants. Overall, our results suggest that Arctic-alpine species can serve as models to understand species responses to climate change. Our reconstruction suggests that *A. alpina* has been struggling with increasing temperatures since the last glacial maximum, however with some potential to adapt to novel environmental challenges. This adaptive potential is possibly mediated by standing genetic variation for adaptive traits, that is present in local, isolated populations in a fragmented landscape.

TALK

# High within- and between- individual structural variability is related to transposable elements insertions and deletions in bivalves

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Structural variants (SVs) represent one of the most abundant sources of genetic variation across a wide diversity of eukaryotes. The increasing number of high-quality genomes has underscored their pivotal role in species diversification and evolutionary processes. Transposable elements (TEs) stand out as primary contributors to the emergence of SVs, because of their mobility and repetitive nature. Oysters, an economically important clade of bivalves, host a rich and diverse TE landscape. Despite their potential impact on genome evolution and diversification, TEs remain relatively understudied. In this study, we analyzed four high-quality oyster genomes spanning the Ostrea and Crassostrea genera together with comprehensive population dataset for the Estuarine oyster *C. ariakensis* to characterize within- and between-individual SVs and their relationship with TEs. After extensive simulations and benchmarks, our findings revealed that up to the 10% of oyster genomes exhibit within-individual structural variability in terms of insertions and deletions between homologous chromosomes. These variants are significantly enriched in TEs, with transposon insertions that outnumber genomic deletions. Furthermore, different TE lineages are active among distinct *C. ariakensis* populations and derived SV segregate among distinct *C. ariakensis* populations similarly to SNPs. This might suggest a possible crucial role for SVs and TEs in facilitating local adaptation phenomena. This study represents the first effort to elucidate the role of SVs in shaping bivalve genome evolution, offering empirical evidence of the successful integration of both long and short reads technologies in compiling a high-fidelity set of SVs and de novo TE insertions useful for population genomic analyses in non-model species.

TALK

# Methylation and ageing in King penguins

Flavia Nitta Fernandes[1], Mikaela Hukkanen[1], Simon Jarman[2], Leyla Davis[3], Maria Elena Figueroa[4],
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Age structure is a fundamental parameter in studies of population dynamics and wildlife management. Yet, when no long-term monitoring studies are in place, as in the case of most wild species, obtaining age information from individuals of wild populations is not a straightforward task. Owing to the advancement and accessibility to next generation sequencing technologies, age estimators based on DNA methylation levels, known as epigenetic clocks, have gained popularity in the last decade. Epigenetic clocks grant valid information about the ageing process, as age is highly correlated to methylation patterns in CpG sites (i.e., a cytosine nucleotide followed by a guanine nucleotide in the 5’ to 3’ direction of the DNA strand). Here, we made use of a long-term (>20 years) monitored King penguin (*Aptenodytes patagonicus*) wild colony to build an epigenetic clock for this long-lived avian species. We detected CpG loci that were correlated with chronological age according to both Pearson and Spearman tests in 96 individuals sampled at different time-points. We designed bisulfite-primers for the top CpG-sites in the correlation analysis to develop a multiplex-PCR assay to measure methylation levels at these sites. The sites that had primers that were found to be functional in the multiplex assay were used to calibrate the epigenetic clock. We aimed to design the primers for regions that were conserved throughout the bird phylogeny, using a multispecies alignment of 364 species and phyloP scores. Our final objective is to generalise this clock to other species, as well as to apply it in other King penguin breeding colonies which are not currently under long-term monitoring, such as a newly established colony in Tierra del Fuego in Chile.

TALK

# Genomic variation along replicate environmental gradients reveals parallel patterns of local adaptation in wall lizards

Stephanie Sherpa[1], Viola Di Canio[1], Martina Muraro[1], Benedetta Barzaghi[1], Andrea Dalpasso[1], Mattia Falaschi[1], Benedetta Gambioli[2], Raoul Manenti[1], Silvio Marta[1,3], Paolo Momigliano[4], Emanuele Berilli[5], Elia Lo Parrino[1], Stefano Scali[6], Federico Storniolo[7], Leonardo Vignoli[1], Marco A. L. Zuffi[8], Roberto Sacchi[7], Daniele Salvi[5], Gentile Francesco Ficetola[1],[9]

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Evolution has generally been considered to be unpredictable because genetic variations in the genome are known to appear randomly, and their incorporation in the population also depends on the balance between the strength of migration, genetic drift and selection. Nonetheless, several evidences of parallel phenotypic changes suggest that evolution can repeat itself in independent populations subjected to similar selective challenges. However, the lack of insight into the causes for repeatability of evolution impedes any progress in predicting the response of populations to ongoing environmental changes. In this study, we specifically ask the drivers of adaptive patterns and the extent of evolutionary repeatability (population size, drift-migration model, environmental gradient width). We collected populations of the Italian wall lizard, *Podarcis siculus*, along replicated climate and urbanization gradients in multiple islands. Using high-throughput sequencing techniques to detect signatures of selection in each island, we found both identical and different genetic changes to similar environmental gradients. Similar adaptive patterns in multiple islands were found in 149 genes at different levels, from repeated single mutations to distinct mutations in the same gene. Most of the genetic changes were unique to islands and their chromosomal distribution varied between islands. Still, gene set analysis revealed that overrepresented biological functions were consistent across islands. The absence of relationships between the levels of unique or shared signatures of selection and neutral genetic variation suggests that adaptive patterns are not determined by demographic history or the drift-migration balance. This study shows that local adaptation lies along a spectrum of population trajectories, but the detection of identical gene-environment relationships in multiple islands, despite different population histories, suggests that parallel evolution has a key role in the adaptation to replicate environments.

POSTER

# Investigating the Italian genetic variation through time with ancient complete genomes

Rajiv Boscolo Agostini[1], Stefania Vai[2], Maria Teresa Vizzari[1], Silvia Perretti[1], Federico De Pizzol[1], Alissa Mittnik[3,4,5,6,7], Donata Luiselli[8], Olga Rickards[9], Luca Sineo[10], Nicoletta Volante[11], David Emil Reich[3,4,5,6,12,13], Silvia Ghirotto[1], David Caramelli[2]

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Italy's unique location in the heart of the Mediterranean Sea and its diverse environments and climates have played a significant role in its history and prehistory. As a peninsula, accessible from both the European mainland and the sea, Italy has served as a crossroads for migration, trade, and cultural exchange for thousands of years. This has resulted in genetic contributions from across Eurasia, with various populations and cultures mixing within the peninsula and its islands. To explore these complex dynamics, we analysed 43 new ancient whole genome sequences from 16 Italian necropoleis from the Neolithic to the Middle Ages and compared them with a dataset of 292 published genomes. We employed different methodological approaches in order to efficiently analyse these low-coverage genomes, such as discarding heterozygous to obtain pseudo-haploid sequences, or the imputation of missing regions. These alternative approaches allowed us to perform exploratory analyses and observe the distribution of genomic variability in different time transects. Furthermore, we investigate population genetic relationships and the genomic structure of our data and identify ancestral genetic components that contributed to the Italian genetic makeup. Our results show the role of specific external contributions, such as different levels of genetic contributions from populations of Iran and Pontic Steppe from Chalcolithic to the Iron Age. Our findings shed light on the intricate genetic mosaic of ancient Italy, revealing how diverse populations have shaped its genetic landscape over millennia.

POSTER

# Evolutionary insights from long-read sequencing data in different barn swallow populations and subspecies

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The barn swallow (*Hirundo rustica*) is an iconic long-distance migratory songbird with six described subspecies that differ in body size, ventral coloration, tail feather length, and migratory behavior. Taking advantage of the chromosome-level, karyotype-validated reference genome recently generated by our research group for this species, we are conducting a resequencing project involving individuals from two migratory European populations (*H. r. rustica*) and a sedentary Israeli population (*H. r. transitiva*). Using long-read sequencing technology, we identified both single nucleotide polymorphisms (SNPs) and structural variants (SVs) and analyzed genetic variability within and between populations. This is to our knowledge the first curated, long-read based SVs dataset generated for this species. We found low genetic differentiation, with the two European populations as a panmictic population. Genome-wide selection and differentiation scans using both SNPs and SVs pointed to candidate genes previously implicated in growth and photoperiod-induced response. Several SVs were associated with candidate genes involved in the above phenotypes, along with singing behavior and abdominal body fat accumulation. The analysis of SVs allele frequency plots suggests their influence on fitness. Multiple morphological, reproductive, and migratory data are available for all samples. We are currently conducting genome-wide association studies to identify genes controlling key life-history traits, with the ultimate goal of better understanding the genetic control of various migratory, phenotypic, and life-history traits, and to assess whether the genetic variability present in this species may be sufficient to cope with the challenges of near-term climate change.

POSTER

# Population dynamics of the fin whale (*Balaenoptera physalus*) in the Sea of Cortez and the Mediterranean Sea inferred from whole genome sequencing

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Defining the factors that influence gene flow in the marine realm can be challenging, especially in pelagic environments. Among pelagic species, cetaceans have some of the highest dispersal capacity and range breadth, so it might be expected that even very distant populations could be genetically related. Conversely, many cetaceans often show substantial genetic structure on a regional or even fine scale level. The fin whale, *Balaenoptera physalus* (Linneaus, 1758), is a species of baleen whales within the parvorder of Mysticeti and the second largest species on Earth. It is a species of cosmopolitan rorquals, although its global distribution is not fully known. Fin whales, like most mysticetes, typically undertake long-range annual migrations. As an exception, resident populations of fin whales have also been documented. Currently, research studies have mainly focused on two year-round resident populations: the population of the Sea of Cortez (Gulf of California, Mexico) and the population of the Mediterranean Sea. The Gulf of California fin whales' population has been characterised by several bioacoustic, telemetry and genetic studies as a resident population. On the other hand, the Mediterranean fin whale population has been poorly characterised from a genetic point of view, and only a few studies based on traditional molecular markers have been conducted to determine whether this population is genetically isolated. The aim of this work was to investigate the phylogeography and population structure of two year-round resident and stable populations using a whole-genome sequencing approach, with a main focus on the Mediterranean population, whose dynamics are currently poorly studied. Our analyses based on whole-genome data of 38 individuals (N = 13, Mediterranean Sea; N = 7, Gulf of California; N = 18, North Atlantic) confirmed the genetic isolation of the Gulf of California population. Moreover, we detected a degree of differentiation within the Mediterranean population that was previously undetected using traditional molecular markers.

POSTER

Eligible for Best Poster Prize

# A population genomics approach to study the structure and evolution of native and introduced populations of the mountain wood ant *Formica paralugubris*

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*Formica paralugubris* is a member of the red wood ants (*Formica rufa* group) native of the western Alps. These species are extremely important for the forest ecosystem functioning, owing to nutrient recycling, seed dispersal, soil aeration, and predation. Because of the intense predatory activity on other arthropods, between 1950 and 1980 there were numerous introductions of *F. paralugubris* in the Apennines, in Italy, as biocontrol agents against forest insect pests. Here we present the first draft of the complete, high-quality genome of this species and preliminary genomic data to assess the genomic diversity across its native and introduced ranges. Moreover, historical museum samples, from the original locations of transplantation, dating back to 1960s, as well as present-day samples from native and introduced populations are being analyzed to further shed light on the genetic diversity of Italian *F. paralugubris*. This approach will allow us to assess the evolution in space and time of this species and possibly to identify demographic and adaptive signatures that may be linked to its biology.

POSTER

# Unlocking the secrets of evolutionary fitness and adaptation in King penguins

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Across the millions of genetic polymorphisms that reside within most species, our understanding of their connection to fitness and adaptive evolution remains limited. Leveraging ecological data from a long-term monitoring program, a newly generated chromosome-level genome assembly, and trio data to estimate mutation and recombination rates, we are using the King penguin (*Aptenodytes patagonicus*) as a long-lived vertebrate model to explore the genomic determinants of fitness and adaptation. In its simplest definition, evolutionary fitness involves the ability of organisms to survive and reproduce. Our first study aims to dissect these components by quantifying polymorphisms related to reproductive fitness. We are analysing the genomes of long-lived (≥ 13 years) King penguins with high reproductive success (n = 32) and low reproductive success (n = 32) to understand the role of genetic diversity and genetic load in determining fitness. Additionally, by examining individuals sampled at three timepoints (2001, 2008, 2020) (n = 84), we are employing temporal genomics to identify genomic regions associated with rapid adaptation. Ultimately, our research will provide critical insights into the genetic basis of reproductive success and adaptive evolution, elucidating the evolutionary processes that shape fitness and resilience in a wild long-lived vertebrate.

POSTER

Eligible for Best Poster Prize

# Robust inference of phenotypic traits from low-coverage ancient genomes

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The forensically validated HIrisPlex-S system represents the most popular model for the simultaneous prediction of eye, hair, and skin colours. Based on the analysis of 41 pigmentation gene polymorphisms, it allows the estimation of individual probabilities for three eye, four hair and five skin colour categories by analysing only genotypic data. This tool was primarily developed to aid specific criminal investigations, in cases with inconclusive STR analysis, but, in the last decade, many studies have aimed to predict the phenotype of ancient human skeletal remains using the well-validated HIrisPlex-S system. There are several aspects that forensic DNA analysis and studies of ancient DNA (aDNA) have in common, such as DNA degradation that results in loss of data. The aim of this study is to test the robustness of the HIrisPlex-S system when applied to low-coverage ancient data, and to evaluate the power of this phenotypic inferential procedure when dealing with methods that explicitly take into account the uncertainty of the genotype calling in low-coverage data, or relying on imputation to estimate genotypes. The final purpose is to provide an operating manual for the inference of phenotypic traits from low-coverage and ancient genomes. Based on our guidelines, we inferred the geographical distribution of human eye, hair, and skin pigmentation on 459 Eurasian individuals, spanning from the Upper Paleolithic to the Iron Age.

POSTER

Eligible for Best Poster Prize

# Exploring the phenotypic correlates of a massive chromosomal inversion in the lesser kestrel (*Falco naumanni*)

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Chromosomal inversions are now recognised as playing a key role in multiple evolutionary processes, such as speciation, adaptation, and sex chromosome evolution. Large inversions, that link together several genes, are frequently associated with complex phenotypic and behavioural polymorphisms. We identified a 57Mb inversion polymorphism in a small migratory falcon species, the lesser kestrel (*Falco naumanni*), encompassing > 40% of Chromosome 2 (~5% of the genome) and containing > 500 genes. We characterised the inversion by genotyping 90 unrelated adults across the species breeding range using whole-genome sequencing and double-digest restriction site-associated DNA. The inversion occurs at high frequency across the Eurasian range and shows no association with geography. Inversion haplotypes are highly divergent, with a divergence time estimated at ~2 Mya. Between the two haplotypes, we identified 148 genes with potentially high-effect mutations. To identify morphological, behavioural, phenological and fitness-related traits associated with the inversion, we genotyped the inversion polymorphism in 351 adults and 316 juveniles from a large breeding population in southern Italy (Matera). We found no deviations from Hardy-Weinberg equilibrium, suggesting that this inversion might not be currently under selection. Furthermore, we found no significant associations between the inversion genotypes and any of the analysed traits. Our results highlight the challenges in characterising the phenotypic basis and the mechanisms that maintain large inversion polymorphisms in natural populations, even when high numbers of mutations have accumulated in protein-coding genes.

POSTER

Eligible for Best Poster Prize

# Pangenome-based Inference of Human Population History

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Over the past decade the field of genetics and genomics experienced an unprecedented change which has revolutionized how we acquire and process bioinformatic data. Along with long-read sequencing, a decisive factor in this transition has been the design and implementation of new algorithmic approaches and data structures. Earlier last year, the first draft human pangenome reference was released thanks to the collective effort of the Human Pangenome Reference Consortium with the objective of integrating and improving over the linear reference. This data structure already contributed to better represent and characterize novel structural variants, but also to substantially reduce the false positive discovery rate of small variants. As part of this project, we leveraged the human pangenome to investigate the demographic history of human populations. We focused on obtaining an unbiased set of genetic markers to explore those demographic scenarios which have proven difficult to ascertain based on traditional strategies. Consequently, we selected short-read data for samples from the Simons Genome Diversity Project that, albeit smaller in size compared to other large scale sequencing programs, encompasses a greater diversity of geographically distributed populations. Reads alignment and genotyping have been performed with cutting-edge graph alignment algorithms - vg Giraffe - and variant callers - Deep Variant, respectively. After preliminary assessments of the samples' genetic diversity through dimensionality reduction, we estimated the likelihood of different, although non-mutually exclusive, migratory patterns with Approximate Bayesian Computation and determined their associated evolutionary parameters with Random Forest Classifier. In conclusion, the results of this study from one side will corroborate the existing evidence on the apportionment of human genetic diversity, from the other they might help shed light on our evolutionary history with unprecedented resolution.

POSTER

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

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Uncovering the dynamics promoting the onset and perpetration of social inequalities in prehistoric societies is a major challenge. We now have the opportunity to exploit archaeogenomic data to describe, with unprecedented resolution, past population structure and processes and to shed light on sociocultural dynamics (e.g., the lineage-based transmission of social status and wealth) which are the basis of the onset of inequalities. Two major population dynamics within the last 10,000 years 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 about the impact of these migrations at the level of single societies. In this project, we propose a high-resolution multidisciplinary study of three burial sites in Northeast Italy from the Neolithic, Eneolithic, and Bronze Age periods to infer the social and genetic structure and their possible change in this time transect. We will sequence whole genomes of 110 individuals to provide a detailed reconstruction of biological relatedness that, combined with a deep resolution chronology, the fine assessment of grave goods and burial practice, individual mobility and dietary resources access provided by stable isotopes analysis, will give us information about the presence of inequality between the members of each burial site. The results of this study will help us to shed light on the onset of social inequality in Northern Italy, and on the cultural and biological mechanisms that promoted its development.

# Miscellaneous

TALK

## Independent evolution of intracellularity in a host-associated bacterial lineage

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Rickettsiales are a broad and alphaproteobacterial lineage characterised by an obligate and ancient association with eukaryotes. Their host spectrum and effects on the host are highly variable, ranging from vector-borne pathogens, parasites of host reproduction, and necessary mutualists. Most Rickettsiales are associated with diverse aquatic protists, and these still under-investigated associations are fundamental for understanding the evolution of Rickettsiales. According to traditional hypotheses, the Rickettsiales ancestor was an intracellular bacterium, similarly to the few thoroughly investigated representatives, but this view has been recently challenged. First, intracellular localisation and host cell entry mechanisms are highly variable in Rickettsiales. Moreover, a fully extracellular Rickettsiales bacterium was discovered, being still host-dependent, but to a significantly lesser degree, thanks to a much wider biosynthetic repertoire. In this work, we aimed to evaluate alternative hypotheses on the evolution of Rickettsiales with a comprehensive approach. Thus, we obtained a wide and representative genomic dataset of Rickettsiales (113 organisms, mostly from different genera), by sequencing eight selected Rickettsiales (seven novel genera) associated with protists, and by further mining of metagenomic datasets to identify assemblies belonging to known Rickettsiales families as well as to novel Rickettsiales-related lineages. Thus, we performed detailed reconstructions of their functional repertoires and their evolutionary variations. We found evidence of multiple independent instances of horizontal acquisitions of membrane transporters in different Rickettsiales sublineages, allowing the progressive loss of biosynthetic pathways for nucleotides, amino acids and other metabolites, thus leading to distinct conditions of host-dependence in each sublineage. Moreover, we reconstructed that, starting from an ancestral arsenal of interaction apparatuses, each clade has undergone a differential evolutionary path, including the lineage-specific development of specialised molecules involved in host cell adhesion and invasion. Accordingly, we conclude that obligate host-association, as well as obligate intracellular evolved “late” and in parallel in different Rickettsiales sublineages.

TALK

# The Evolution and Characterization of the RNA Interference Pathways in Lophotrochozoa

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In animals, three main RNA interference mechanisms have been described so far, which respectively maturate three types of small noncoding RNAs (sncRNAs): miRNAs, piRNAs, and endo-siRNAs. The diversification of these mechanisms is deeply linked with the evolution of the Argonaute gene superfamily since each type of sncRNA is typically loaded by a specific Argonaute homolog. Moreover, other protein families play pivotal roles in the maturation of sncRNAs, like the DICER ribonuclease family, whose DICER1 and DICER2 paralogs maturate respectively miRNAs and endo-siRNAs. Within Metazoa, the distribution of these families has been only studied in major groups, and there are very few data for clades like Lophotrochozoa. Thus, we here inferred the evolutionary history of the animal Argonaute and DICER families including 43 lophotrochozoan species. Phylogenetic analyses along with newly sequenced sncRNA libraries suggested that in all Trochozoa, the proteins related to the endo-siRNA pathway have been lost, a part of them in some phyla (i.e. Nemertea, Bryozoa, Entoprocta), while all of them in all the others. On the contrary, early diverging phyla, Platyhelminthes and Syndermata, showed a complete endo-siRNA pathway. On the other hand, miRNAs were revealed the most conserved and ubiquitous mechanism of the metazoan RNA interference machinery, confirming their pivotal role in animal cell regulation.

TALK

# The cellular mechanism of aging from hypothesis to scientific evidence

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There is a main difference between theories explaining aging as a genetically determined and modulated adaptive phenomenon and theories explaining aging as a non-adaptive phenomenon caused by the accumulation of random degenerative events. In fact, for adaptive theories a genetically determined and modulated program determining aging is indispensable, while for non-adaptive theories such a program cannot exist. However, the evidence supporting the existence of this program appears strong according to the mechanism of the subtelomere-telomere theory of aging with the action of TERRA sequences. This theory was developed in four successive phases: 1) Aging simply caused by limitations in cell duplication; 2) Aging caused by progressive telomere shortening; 3) Aging caused by progressive inhibition of particular hypothetical regulatory sequences in subtelomeric position (r-sequences) determined by progressive telomere shortening; 4) Identification of the r-sequences in the TERRA sequences whose effects are well known and documented. The theories 1 and 2 were untenable because their predictions were contradicted by the evidence. Theory 3 was based, among other things, on the hypothesis of sequences that had to be confirmed by evidence. Phase 4 overcame this difficulty. The mechanism proposed by theory 4 describes a determined and regulated genetically pivotal mechanism of aging, and therefore confirms the hypothesis of aging as an adaptive phenomenon and invalidates the opposite thesis. Among other things, for the validity of the hypothesis of aging as a non-adaptive phenomenon, it would be essential to justify in evolutionary terms: (i) the position of regulatory sequences of great importance for cellular functions in a position where they are inhibited by telomere shortening; (ii) cell senescence which is an oncogenic factor and cannot be explained as a defense against cancer; and (iii) gradual cell senescence which cannot be hypothesized as an anti-cancer defense. Furthermore, the phenomena referred to in points (ii) and (iii) and TERRA sequences inhibition are reversible with appropriate manipulations and this is incompatible with their possible interpretation as a consequence of random degenerative phenomena.

TALK

# Exploring selective pressures acting on genes involved in immunity in Malagasy mantellid frogs

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Positive selection stands at the base of adaptive evolution by fixing over time a beneficial genetic mutation into a population. A measure to assess the selective pression occurring on sites, proteins or genes is a parameter known as dN/dS ratio, calculated as the ratio between the non-synonymous and synonymous substitution rate. According to its estimated value, it shows evidences of negative (<1), neutral (=1) or positive (>1) selection, which allow to understand whether selection acting on that part of the genome is the likely source of new adaptive traits or whether it encodes traits that will likely be conserved. In this work, we explored the nature and strength of selective pressures acting on differentially expressed genes involved in the immune system functions in Malagasy frogs of the subgenus Brygoomantis (Anura: Mantellidae: Mantellinae: Mantidactylus). The dataset is composed by genes which were found to be differentially expressed in the liver and in the skin and that Gene Ontology analyses suggested are related to immune system functions. The identification of these genes in Malagasy frogs is here documented for the first time. These genes can have an important role in the immunological responses to pathogens, one of the main treats to amphibians worldwide. We found evidences of positive selection acting in the immunological genes from skin and liver here analyzed, suggesting a possible role of immune system in driving the speciation process of Brygoomantis subgenus.

TALK

# Conservation of the oscillatory genes among the marine angiosperm *Zostera marina* and land plants: a comparative approach

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The physical movements of celestial bodies, including the Earth rotation around its axis and its revolution around the Sun, generate natural cycles of approximately 24 hours governed by light/dark alternation. As adaptation to the life on our planet, almost all the living organisms shape their physiology, behavior and development on the natural transition between light and dark periods. Either the temporal alignment of biological processes to the natural cycles and their anticipation are coordinated by an endogenous timekeeper named circadian clock. In plants, changes in photoperiod are perceived by light receptors which transfer the input signals to the central core clock oscillators. In turn, the clock regulators may activate molecular responses involved in different biological processes. In this work, we generated RNA-seq data over 48 h periods in light-dark (LD) and constant light (CL) conditions from the marine flowering plant Zostera marina. These data were compared with already available transcriptional datasets coming from three land plant species (i.e. *Arabidopsis thaliana*, *Oryza Sativa* and *Wolffia Australiana*). In order to detect the oscillatory profiles in the selected species, we applied two algorithms specifically designed to identify the oscillatory patterns in large-scale datasets, i.e. JTK_CYCLE and BioCycle. Then, to infer about the orthology and the paralogy relationships existing among the rhythmic genes detected in the species of interest, we used a comparative genomics approach based on sequence similarity. Globally, these analyses revealed that the percentages of circadian regulated genes are lower in *Z. marina* with respect to the other species, from 33% in *A. thaliana* to 4,9% in *Z. marina* (CL condition). Moreover, we found also differences in the expression patterns of orthologs/paralogs among the four plants lineages. This study will shed light on the evolution of the rhythmic genes in marine environment, which is not yet defined.

TALK

# Unraveling the Function and Evolution of Retinoic Acid Signaling with the Mollusk *Mytilus galloprovincialis*

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Vitamin A (retinol) and its derivatives play crucial roles in metazoan, primarily through the active metabolite all-trans retinoic acid (RA), which, in vertebrates, modulates transcription via the retinoic acid receptor (RAR). While the evolution of RA signaling remains largely unknown, mollusks RARs seem to have lost the ability to bind RA due to mutations in key amino acids of the ligand-binding domain (LBD). Although mollusks can produce RA, its biological role remains elusive, and it is also unclear whether RAR contributes to RA-dependent signal transduction. In this study on the bivalve mollusk *Mytilus galloprovincialis* (Mg), we screened its pan-genome and identified orthologs of RA signaling-related genes. Subsequent sequence and computational analyses of the MgRAR LBD revealed amino acid signatures suggestive of a reduced responsiveness to RA, which was confirmed by in vitro assays demonstrating an absence of its activity. However, exogenous RA treatment during embryogenesis resulted in specific phenotypes, and a differential transcriptome analysis revealed a significant upregulation of a Cyp26 gene, encoding an RA-metabolizing enzyme, supporting a negative feedback mechanism not previously reported in lophotrochozoans. A physiological role of CYP26 was demonstrated by treating embryos with both RA and a CYP26 inhibitor, which induced phenotypic alterations resembling those caused by high RA doses. Ongoing analyses will determine if Cyp26 is directly regulated by RAR or if other nuclear receptors, such as RXR, mediate its activation and therefore RA signaling. *M. galloprovincialis* thus represents a valuable model for elucidating both the function and the evolution of RA signaling in metazoans.

TALK

# Evolution of West Nile virus between Europe and Africa

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West Nile virus (WNV) is an arthropod-borne virus that mainly infects mosquitoes and birds, but can often spread to other hosts such as horses and humans, causing mild to severe disease, and even death. WNV was identified for the first time in Uganda in 1937 and later emerged in many different areas of the world, including North America and Europe. In this latter area, 1,133 human cases, with 92 deaths, were registered for the 2022-2023 season alone, and the virus is constantly detected in many European countries. Despite this burden, the evolution and transmission dynamics of WNV within Europe and between Europe and Africa haven't been clarified yet. For this reason, we assembled a database of more than 500 WNV genomes, comprising all publicly available genome data, and sequenced 75 new genome sequences from Italy and Senegal. To track the spread of the virus in space and time, we used continuous phylogeography and molecular clocks, taking advantage of a manually curated database with all dates and locations for each sample. We found different evolutionary dynamics for the two main pathogenic lineages of the virus, WNV Lineage 1 (L1) and WNV Lineage 2 (L2). WNV L1 is characterized by continuous introductions from Africa to Europe, moving from West Africa to Spain, France, and Italy, with occasional reintroductions in the opposite direction. Conversely, WNV L2 arrived in Europe from southern Africa after a few introductory events and later spread across the continent, becoming endemic in many countries such as Italy, Hungary and Greece. Our study clarifies the emergence of WNV between Africa and Europe, representing a useful resource for implementing new health policies and surveillance strategies. We call for increased genome sampling in both continents to enhance the resolution and precision of outbreak reconstructions, which will provide useful information to contain the virus and predict its further spread, helping to save human lives and economic resources.

POSTER

# Investigating the origin and recolonization dynamic of the Eurasian otter (*Lutra lutra*) at the boundary of its Italian core range through non-invasive genetics

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During the past century the Eurasian otter (*Lutra lutra*) suffered a drastic decline throughout the rivers of the Italian peninsula, surviving only in Southern Italy. Recent surveys have revealed irrefutable signs of otter expansion in Central Italy and, specifically in the last 15 years, along rivers of Abruzzo region. Gradually expanding along the Sangro river, otters have also recolonized the Abruzzo, Lazio and Molise National Park (PNALM), an area fundamental for the persistence and expansion of the species to the north. To explore both the dispersal dynamic from Southern Italy to Abruzzo and the potential factors that may limit gene flow at the boundary of the Italian core range, particularly between the population from the PNALM and the surrounding areas, we genotyped faeces (spraints), anal jellies and tissue samples from Central and Southern Italy. In this regard, DNA was extracted from 52 specimens from the regions of Abruzzo, Molise, Campania, Apulia and Basilicata, and used to amplify a panel of 11 microsatellite loci. Results revealed a significant genetic differentiation between Southern and Central Italy, suggesting a stepping-stone recolonization process. Genetic structure analysis and gene flow estimation suggested a complex pattern of recolonization of the Abruzzo region, possibly originating from a southern source population, with a dispersal path not following a single trajectory northward. Particularly, the similarity observed among our coastal samples suggests the presence of a seacoast corridor along the Adriatic side of the Italian peninsula. Population structure in Abruzzo is weak and genetic diversity low, consistent with a founder effect. The effective population size (Ne) estimate ($7.3<Ne<17.8$) could indicate the presence of few breeding adults in the PNALM, but census population size (Nc) estimate ($Nc=50$) and the 18 unique genotypes, suggested the hosting of a higher number of individuals than initially assumed, likely made up of different demes, as indicated by sibship analysis. Although otter nucleus in PNALM is showing a favourable conservation status, its genetic diversity is low, making it vulnerable to demographic and environmental stochastic factors. Accordingly, interventions are recommended to favour movements of otters and increase the likelihood of stabilization and expansion of Central Italy's population.

POSTER

Eligible for Best Poster Prize

# Unraveling the Evolutionary Drivers of P450 Diversity in Polyneopteran Insects

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The broad and functionally diversified superfamily of enzymes known as cytochrome P450 plays a pivotal role in the metabolism of a wide range of substrates, including fatty acids, steroids, and xenobiotics. These enzymes serve an essential part in biological molecule production, harmful substances detoxification, and metabolic adaptation to environmental changes. Understanding the variables that contribute to the molecular diversity of P450 proteins is crucial for elucidating their evolutionary history and functional diversification. Leveraging a comparative genomic framework, we investigated this topic in Polyneopterans, a group of insects with an extensive variety of life features and ecological niches, which provided the ideal testing ground for this study. Utilizing a novel pipeline for characterizing large and diverse gene families from unannotated genomes, we achieved unprecedented scale in the number of genomes and in the characterization of more divergent gene family members. We explored how ecological factors and the resulting adaptive pressures shape the P450 repertoire across this insect group. By integrating ecological data with our molecular findings, we identified correlations between specific environmental challenges and adaptive changes in P450 genes. Furthermore we tackled one of the more well-known principles of phylogenomics, the Orthology Conjecture, which states that orthologs are more likely to retain their original function than paralogs. Specifically we assessed whether gene duplication or speciation events contribute differently to selection regimes and P450 sequence evolution, here used as proxy of divergence from the ancestral function. Our findings provide new insights into the evolutionary mechanisms driving the diversification of this crucial enzyme family.

POSTER

Eligible for Best Poster Prize

# Genomic Insights into the Evolution of Eusociality in Hymenopterans

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Eusociality is a complex phenotype and a remarkable evolutionary innovation that strongly characterizes the Aculeata clade within the suborder Apocrita of the hymenopterans. Eusocial species probably comprise less than 2% of hymenopterans, but this single evolutionary driver has resulted in their disproportionate contribution to global insect biomass. Despite this success, defining the exact line between eusocial, social, and non-social phenotypes can be challenging, highlighting the nuanced nature of social evolution within this order. To unravel the genetic underpinnings of this remarkable evolutionary leap, a comparative genomic approach was used to investigate the genetic basis of eusociality across eighty-one hymenopteran genomes. Signals of convergent evolution were searched by applying analyses based on relative evolutionary rates using the software TRACCER. Investigating genes that exhibit stronger evolutionary signals in social lineages made it possible to pinpoint specific genetic pathways potentially targeted by selection during the evolution of eusociality. The analyses revealed compelling molecular signatures underlying the emergence of sociality, which appear to be associated with a solid genetic background and potentially with a high evolvability of different pathways to eusociality.

POSTER

Eligible for Best Poster Prize

# A new potential symbiont for Cryptocephalinae beetles (Coleoptera, Chrysomelidae): prevalence in the subfamily, localization in the insect body and functional role for the host

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Studying the interactions between phytophagous insects and the associated bacteria is of scientific interest for both pure and applied research. Bacteria can provide insects with amino acids, cofactors, and vitamins lacking in their diet, along with functional traits allowing them to exploit specific diets and colonize different ecological niches. An exemplar case of phytophagous insect-bacteria association is represented by the leaf beetles (Coleoptera, Chrysomelidae), where both primary symbionts and other bacteria of the gut microbiota facilitate the degradation of recalcitrant plant tissue compounds. In a previous study characterizing the bacterial communities of Chrysomelidae, a bacterium of the family Morganellaceae (Enterobacterales) was detected in *Cryptocephalus bipunctatus* and *Macrolenes dentipes* (Cryptocephalinae) and identified as a possible symbiont for these species. The main aims of the present study are thus to evaluate if other species of the subfamily Cryptocephalinae host this bacterium, locate it within the insect body and determine if it has a functional role for the host. Currently, the DNA from 55 different Cryptocephalinae species has been extracted and the full 16S rRNA gene has been PCR amplified using specific primers. The obtained amplicons were sequenced to confirm the symbiont's presence and analyze its 16S rRNA nucleotide variability. Ten species of Cryptocephalus were found positive to the symbiont. Among them, *C. flavipes*, reared at laboratory conditions, revealed the symbiont presence in the eggs laid by positive adults and in the first-instar larvae, suggesting vertical transmission through generations. Future experiments will aim to locate the symbiont within the insect body by using fluorescence in situ hybridization. Furthermore, the genome of this bacterium will be sequenced to evaluate its potential to provide the host with functional traits that enhance adaptation to the living environment.

POSTER

Eligible for Best Poster Prize

# Origins and implications of mitochondrial genes expansion in bivalves.

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Bivalvia are a class of molluscs with various oddities in their mitochondrial genomes, which are partly associated to the peculiar mitochondrial segregation mechanism that is exclusive to this group. Many of them host a large number of unassigned regions and elongations that are uncommon in animal mitochondrial genomes. To re-evaluate the annotation and function of mitochondrial proteins in bivalves, we established a comparative approach, paying particular attention to nuclear OXPHOS compatibility and gene boundaries. Since mutations involving start and stop codons can significantly affect the size of a gene, we initially investigated the genesis of extensions by assessing oversupply and lack of such codons throughout genomes. Next, we used AlphaFold and related scoring methods to determine the potential influence of elongations into OXPHOS core proteins on their interactions with nuclear-encoded subunits. In this work, we share some insights into our attempts to re-examine the mitochondrial genetics of bivalves. Our goals are twofold: to give a comprehensive assessment of the group's mitochondrial gene features and to elucidate the causes, effects, and conservation of mitochondrial expansion in metazoans.

POSTER

# Mycosium: sporulating knowledge. Why fungi can offer us new perspectives on evolution

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Within Eukaryotes, fungi are characterized by some of the most spectacular examples of evolutionary adaptation; indeed, life on Earth would look very different in the absence of Kingdom Fungi. Firstly, fungi are incredible players in global biogeochemistry, recycling carbon and mobilizing nitrogen, phosphorus and other bio-elements. Saprotrophs are the most specialized decomposers of highly recalcitrant organic matter, such as lignin. Fungi provide essential support to plant life in the form of endophytes and mycorrhizae, while fungal pathogens can decimate plant and animal populations, threatening food supplies. Furthermore, the metabolic singularities of many fungi have provided humanity with fermented foods and beverage to feed us and alter our minds, drugs to cure our bodies, and many compounds with important industrial usages. However, despite their incredible taxonomic and functional diversity, fungi rarely occupy a central position, even at university degrees. In fact, fungal studies are currently neglected in the (Italian) academic system, partly because of the lack of dedicated courses and chairs. As evolutionary biology students passionate about fungi, we felt the need to independently find ways to overcome these lacks and to create a project committed to dissemination and sensibilization in the academic community and towards broad public as well. In June 2023, we successfully organized a two-day symposium with the participation of some of the most important Italian and international researchers of the field. The following success allowed the creation of a network that led to the achievement of many goals, such as the introduction of a mycology course at the University of Bologna, the organization of the first Italian mycology festival (MicoCosmo) and several collaboration requests. In the end, Mycosium aims to take part in the process of full accreditation and dignity of the Kingdom, sadly still a prerogative of botanists.

POSTER

Eligible for Best Poster Prize

# Genomic and evolutionary investigation of the tick-borne pathogen *Ehrlichia muris*

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Ticks are some of the most important vectors of zoonotic diseases for humans as well as for domestic and wild animals. The genus Ehrlichia (Rickettsiales, Anaplasmataceae) includes tick-borne intracellular pathogens which infect macrophages. Among those, *Ehrlichia muris* usually infects rodents, but has also been sporadically reported as a human pathogen. The known geographic distribution of this bacterium ranges from Asia, to the United States and eastern Europe. With the present work, we report the finding of *Ehrlichia muris* in an Ixodes ricinus tick collected from a migratory bird (*Turdus iliacus*) in northern Italy (Bergamo province). After sequencing the genome of our isolate, we aimed to provide new insights into the epidemiology of Ehrlichia and the evolution of its interactions with the host. Considering the host distribution, bird migratory routes and molecular phylogeny of the bacterium, it is likely that the pathogen was transported by this migratory bird to Italy from an area endemic for *E. muris*, such as eastern Europe. Moreover, comparative genomic analyses showed that *E. muris* and other Ehrlichia spp. present copy number variations in two families of outer-membrane proteins (VirB2 and Omp-1/P28), probably due to recent duplication, deletion, and recombination events. These differences are likely a source of variability in surface antigens to evade host immunity, potentially playing a role in host adaptation and specificity. These results highlight the impact of migratory birds on the spread of tick-borne pathogens in non-endemic areas and underscore the need for further investigations on the possible establishment of local transmission in susceptible mammalian hosts, as well as the influence of genetic variation on the evolution of host specificity.

POSTER

# The first high-resolution map of DNA methylation in the genome of the mollusk *Mytilus galloprovincialis* and its role during embryogenesis

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Epigenetic modifications play a key role in regulating gene expression in all organisms. In vertebrates among various epigenetic mechanisms, DNA methylation influences embryonic development and gene expression. As a consequence, differentiated cells develop a stable and unique DNA methylation pattern that regulates tissue-specific gene transcription: a critical mechanism for development. DNA methylation remains poorly investigated in invertebrates and aquatic organisms where embryos develop directly exposed to environmental conditions. Therefore, we decided to investigate the mechanisms underlying this process in the marine bivalve *Mytilus galloprovincialis* and belonging to the phylum Mollusca. After an initial characterisation of mussel development, we investigated the expression and the role of the two main genes of DNA methylation machinery, Dnmt1 (methylation) and Tet (de-methylation) during embryogenesis by using specific inhibitors for DNMT1 and TET. Interestingly, we found that, similarly to other vertebrates and invertebrates, embryos treated with these inhibitors display delayed or arrested development. Then, to understand how DNA methylation occurs in the Mytilus genome and which is it relation to gene expression we carried out a Methyl-Rad sequencing in association with an RNA seq on D-Larva at 48 hpf. In general, we found that Mytilus genome displays a higher methylation within protein coding genes and repetitive sequence, compared with intergenic non-repetitive sequence. Within the gene body we found that the methylation levels were significantly higher in the gene body than in the flanking regions, upstream of the transcription start site and downstream of the transcription termination site suggesting a potential role in transcription initiation and termination. Interestingly, we also found that highly expressed genes generally showed a concomitant higher average level of methylation. The relationship between gene body methylation and gene expression is complex. While no clear linear correlation was found, highly expressed genes generally had higher methylation levels than poorly expressed or non-expressed genes at this development stage. Although this suggests the existence of a link between gene body methylation and functionality, further analysis showed these trends were primarily due to gene presence/absence variation (PAV), rather than transcriptional regulation. This study provides the first insights into DNA methylation in *M. galloprovincialis*, which may provide fundamental information to better understand the complex role played by this mechanism in regulating genome activity in bivalves.

POSTER

Eligible for Best Poster Prize

# Standardization protocols for microsatellites analysis in wildlife conservation and forensic genetics

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Microsatellites are extensively employed in animal conservation and forensic genetics. Despite their widespread use, the lack of standardization systems makes routinary use and interoperability difficult. Drawing inspiration from human genetics, we are developing protocols for animals such as *Aquila chrysaetos*. Identifying 10 informative markers from the literature, we designed a multiplex PCR, ensuring non-overlapping fragment lengths and primer specificity. Primers combination, fragment labeling, and capillary electrophoresis analysis verified the robustness of the PCR. Subsequent optimization focused on the allelic ladder, crucial for accurate electropherogram interpretation. Three protocols were tested for allelic ladder development: (1) successive PCR amplifications with enzymatic purifications, (2) agarose gel-based isolation with reamplification, and (3) plasmid-based allelic collection. The first performs well in terms of development time and cost but may lose alleles, the second performs less well in terms of time and cost but may isolate rare alleles. The third performs well in terms of time and includes all alleles but it is more expensive. While the first two protocols require more optimization, the third method is promising for its efficiency in obtaining rare alleles. Future research will focus on refining these protocols and testing allelic ladders across different laboratories and instruments to validate standardization.

POSTER

Eligible for Best Poster Prize

# The evolution of the P450 gene family in true fruit flies

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In plants, the production of defensive chemical compounds is a common strategy to counteract potential predators. Many insect species have developed adaptive mechanisms to detoxify these toxic compounds through the action of enzyme systems capable of interacting with these substances and promoting their degradation or elimination from the body. Among these, P450 enzymes, also known as cytochrome P450 or CYP, are a large and diverse family of genes found in all living organisms with multiple physiological and biochemical functions. We investigated their importance and evolution in six true fruit flies of the genera Bactrocera, Ceratitis and Zeugodacus that differ in their host plant use. In particular, we studied the evolutionary dynamics of this gene family in terms of expansion and contraction, as well as the role of positive selection in the different paralogues. We further focused our attention on *Zeugodacus cucurbitae*, given its dietary habits which include a significant proportion of cucurbitaceae, which contain cucurbitacins, a toxic substance that these plants use as a defence mechanism. Our results reveal species-specific evolutionary patterns that may indeed reflect adaptation to different food sources.

POSTER

Eligible for Best Poster Prize

# Patterns of colonization of the Western European House Mouse *Mus domesticus* in central Mediterranean islands

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The western European house mouse *Mus domesticus* is a common species worldwide. Its distribution around the world has been strongly influenced by the movement of humans and it is now found in central and western Mediterranean region, northern Europe, the Americas, Oceania and southern Africa. Here, we investigated matrilinear genealogy to infer patterns of colonization in five Italian islands located in the central Mediterranean basin. To do so, mice samples were collected from 29 localities in Sicily and from the islands of Pantelleria, Ventotene, San Domino and San Nicola. The mitochondrial marker D-loop was amplified and sequenced and an alignment of 830 sequences was built using present and available data in GenBank. Subsequently, each of the newly obtained sequence was assigned to one of the 11 D-loop haplogroups already described in previous studies. A total of 55 different haplotypes were identified and only 6 of them have already been detected in previous literature. Of the 11 already known haplogroups, 7 of them are represented in the study area. Sicily appears to be the centre of mitochondrial genetic diversity in the Central Mediterranean with 5 different haplogroups. The origin of this diversity is likely due to the importance of Sicily in marine traffic since the Iron Age. In fact, it is known that the rise of sea faring cultures and their commercial activities allowed the dispersal of many mitochondrial clades across the area. Conversely, smaller islands, such as Pantelleria, San Domino and San Nicola, show a lower genetic diversity, suggesting single founder events. On the other hand, Ventotene, despite its small size, shows 2 different haplogroups not even represented in mainland Italy. Therefore, two separate ancient colonization waves are likely to have occurred on the island. These results indicate once again how insular populations of mice in the Mediterranean have been shaped by human activity and ship traffic since ancient times.

POSTER

# Genomics shreds the veil: Hepatincolaceae are a Rhodospirillales sublineage adapted to an association with Ecdysozoa

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Hepatincolaceae are a bacterial clade that colonize the intestinal lumen of several arthropods (e.g., wooudlouse and termites) and other ecdysozoans, being associated with the intestinal microvilli. Previous phylogenetic reconstructions, mainly based on single gene markers, consistently placed this lineage of bacteria within the Holosporales, which, on the other hand, are a lineage of intracellular protist-associated bacteria. However, due to the paucity of genome sequences of Hepatincolaceae, more comprehensive phylogenetic analyses, based on the whole genome contents, have never been performed. Moreover, the genomic features of Hepatincolaceae, both from a functional and evolutionary points of view, were poorly investigated. In this work, we sequenced and assembled novel Hepatincolaceae genomes, and, based on a phylogenomic approach, we determined that the phylogenetic proximity between Hepatincolaceae and Holosporales was an artefact due to sequence compositional biases, and that Hepatincolaeae are indeed a distinct and separate clade from them, being affiliated to Rhodospirillales. Given this new evolutionary landscape, we investigated the genomic content of Hepatincolaceae comparing it with Holosporales and their new Rhodospirillales neighbors, and propose a new scenario in which Hepatincolaceae independently evolved their adaptation to host-association with peculiar traits. Indeed, Hepatincolaceae revealed to be significantly more adaptable from a nutritional point of view (also being able to thrive in anaerobic conditions), and being able to exploit a wider array of molecules which are available in the host gut lumen as compared to the intracellular.

POSTER

# Gene expression profiling of a fish helminth (*Schistocephalus solidus*) throughout its complex life cycle. Are parasite's life stages genetically independent?

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Complex life cycles (CLCs) are a widespread but challenging lifestyle among parasites. Parasites with complex life cycles infect multiple hosts in succession. Therefore, they are adapted to exploit different ecological niches by using the information encoded in a single genome. Are the same genes expressed in all hosts, such as when parasite stages exhibit similar tasks, e.g. during growth in intermediate and definitive hosts? Or are alternative gene sets expressed in different hosts? The adaptive decoupling hypothesis suggests that the different stages in a complex life cycle should be genetically independent, such that selection on parasite traits in one host does not affect traits in other hosts. We tested this hypothesis using a tapeworm, *Schistocephalus solidus*, by sampling transcriptomes throughout its complex life cycle (57 transcriptomes from 10 S. solidus stages). Gene expression analysis was used to identify genes that are differentially expressed between hosts (e.g. 1st host vs 2nd host), between parasite functional stages (e.g. 'transmission' vs 'growing'), and between conditions (e.g. 'growing' in 1st host vs 'growing' in 2nd host). Our results show how the gene expression in each stage differs from all the others and there is no positive correlation between stages sharing the same task or the same host. The highest correlation in gene expression and functional enrichment was found in the stages sampled close in time. A lack of positive correlation means that where some genes are up-regulated (or down-regulated) in one stage, the same genes are either not differentially expressed or down-regulated in all the others, even when the stages are from the same host, or performing the same task. This evidence confirms the independent gene expression of each stage, thus, the decoupling hypothesis in parasitic worms, giving more insights on how CLCs have evolved.

POSTER

# Eco-evolutionary mechanisms driving the assembly and dispersal of host-associated reproductive microbiomes in the guppy (*Poecilia reticulata*)

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The mechanisms by which host-associated microbiomes assemble is unresolved and controversial. Microbial ecologists have hypothesized that genetic and environmental processes may explain the characteristics of animal microbiomes. However, how these factors converge to determine microbiome assembly and variation across hosts remains largely elusive. This project will examine aspects of microbial ecology, evolution and animal biology to reveal the ecological mechanisms involved both in short- and long-term establishment, variation, transmission among hosts and function of host-associated microbiomes. The overall hypothesis behind this project is that microbiome assembly is predominantly and temporally driven by adaptation to the external environment, transmission capacity, and colonization history. By combining genomics, mathematical modelling and animal behavioural studies, we will 1) reveal to what extent microbial dispersal through the environment and host-mediated transmission contribute to microbiomes' assembly and 2) identify how environmental heterogeneity drives the establishment, function and evolution of host-associated microbiomes. Moreover, we will identify fundamental principles about how microbiome assembly processes influence host behaviour and physiology. We will use a novel model organism in host-microbiome ecology, the viviparous and internally-fertilizing guppy (*Poecilia reticulata*). This will be a tractable system to study the mechanistic foundations of microbiome assembly, where the ecological processes can be strictly controlled in a lab environment, thus allowing us to unveil the processes governing host-microbe interactions in the aquatic world. This project is innovative as it offers the first comprehensive assessment of the broader rules driving microbiomes' assembly in a fish species throughout host development, providing a greater understanding relevant to most host-microbe interactions.

POSTER

# Identification of novel toxin resistance genes in Drosophila via Experimental Evolution, GWAS, and CRISPR screening

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Insecticide resistance is a widespread challenge for the management of vectors transmitting pathogens and agricultural pests, requiring a better understanding of the genetic mechanisms underlying evolution of resistance. *Drosophila sechellia* is a compelling model for such studies as it naturally evolved resistance to octanoic acid (OA), an abundant chemical of its noni fruit host that is toxic for other insects, including close relatives *D. simulans* and *D. melanogaster*. We have used a multi-pronged strategy to identify genes contributing to OA resistance. We began by experimentally-evolving *D. simulans* strains with higher tolerance to OA, and determined the resulting genetic architecture. To identify specific candidate genes, we integrated this analysis with a genome wide association study of OA resistance in *D. simulans* as well as a genome-wide CRISPR selection screen upon OA exposure in *D. melanogaster* S2R+ cultured cells. We identified four candidates, with diverse predicted molecular and expression properties, and validated their relevance using loss-of-function analysis in D. melanogaster. Two of these genes displayed an increased expression in the experimentally-evolved strains, paralleling their higher levels of expression in *D. sechellia* compared to other drosophilids; transgene-mediate over-expression of one of these in *D. melanogaster* can promote *D. sechellia*-like levels of OA resistance. Our results hint at an adaptive role of these genes in shaping OA resistance both under laboratory conditions and during *D. sechelli*a's evolutionary history. This study emphasizes the power of integration of multiple genetic approaches to identify genes and cellular mechanisms underlying toxin resistance in insects.

POSTER

# Genetic load dynamics in tetraploid and diploid populations

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Polyploidization is a phenomenon where the genome of a taxon undergoes one or more rounds of duplication. This genetic condition leads to significant changes in species' physiology, morphology, and evolution. Despite polyploidy's impact on the evolutionary history of many species, the population genetic theory for polyploids is less well established than for diploids. It remains largely unknown how polyploidy affects the dynamics of genetic load from deleterious mutations and their impact on individual fitness. Studying this deleterious variation is key to conserving polyploid species, as genetic load affects population survival, particularly in small populations. This work aims to compare the dynamics of genetic load components (masked and realized load) and their fitness effects in tetraploid versus diploid populations using forward-in-time genomic simulations. Considering deleterious mutations under partial recessiveness, our simulations show that tetraploids accumulate more genetic load than diploids in both small and large populations, resulting in a greater decrease in tetraploid fitness over time. Under complete recessiveness, the same pattern is observed in small populations. However, in large populations at load-drift equilibrium, the realized load and fitness of tetraploids and diploids are not significantly different. The only scenario where tetraploids suffered less from genetic load effects was in bottlenecked populations under complete recessiveness. Our results highlight the importance of factors such as demography, dominance, selection coefficients, and drift in how deleterious mutations accumulate in diploids and tetraploids. We show that, under specific conditions, the widespread notion that tetraploids suffer less from genetic load does not hold, emphasizing the need for further research on the genetic load in polyploid taxa.

POSTER

Eligible for Best Poster Prize

# Revision of the fossil material attributed to the genus Palaeotherium (Perissodactyla) preserved at the Capellini Geological Museum in Bologna

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The Capellini Geological Museum, founded in 1860 by the geologist and paleontologist Giovanni Capellini, is one of the most important museums in Italy in the field of paleontology, as it houses over 500,000 specimens. Among these are mastodons, giant sloths, cetaceans, and also fossils belonging to Palaeotherium, a genus of equoid of the order perissodactyla that lived in Europe between the middle Eocene and the lower Oligocene. This genus of perissodactyls was studied and revised in the last century, mainly thanks to the work of Franzen (1968). However, subsequent studies have been fragmented, with many specimens still attributed to invalid species. The various specimens, mainly of dental nature, belong to currently valid taxa such as *Palaeotherium curtum*, *Palaeotherium medium*, and *Palaeotherium magnum*. Other specimens have not been revised due to the need for specific study techniques. It is interesting to note that among the specimens studied and attributed to the genus Palaeotherium, there was the small-sized species called *Palaeotherium minus*, a synonym of the species *Plagiolophus minor* (Remy, 2004).

POSTER

Eligible for Best Poster Prize

# Structural variants reveal genome evolution and adaptation mechanisms in a non-model bird species

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Third-generation sequencing technologies permit a more comprehensive analysis of animal genomes, including the overlooked ones of non-model species. The identification of structural variants (SVs), defined as long (> 50 bp) DNA mutations (insertions, deletions, duplications, inversions and translocations), will particularly benefit from the latest long-read sequencing technologies. SVs may have a substantial impact on fitness and a significant role in evolutionary processes. For example, large inversions may be responsible for maintaining different migratory and reproductive strategies, respectively in willow warblers and ruffs. In this study, we focus on the barn swallow (*Hirundo rustica*), an iconic non-model songbird with several subspecies and populations exhibiting distinct morphological and behavioural phenotypes. Using PacBio High Fidelity (HiFi) sequencing technologies, we uncovered different SV features in three barn swallow populations with distinct life-history and behavioural characteristics. We identified insertions and deletions associated with genes involved in circadian rhythm control (known to be key to migration and reproductive switches), vocal control areas (important for singing behaviour and vocal learning with mechanisms similar to humans), abdominal body fat accumulation (principal fuel during bird migration), moult (essential aspect, requiring precise timing and diet), feathers shape (crucial for flight) and color (different in the sampled subspecies). Furthermore, we observed that SVs may influence individual fitness by examining their distribution across the genome and utilising site frequency spectrum (SFS) analysis. Given the availability of much morphological data, we are currently conducting genome-wide association studies with both single nucleotide polymorphisms (SNPs) and SVs with the aim of identifying even more genes that regulate diverse morphological and behavioural traits in the barn swallow.

POSTER

# Testing different de novo assembly pipelines using simulated ancient metagenomic data

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In the last years, the interest in ancient metagenomics, the analysis of complex DNA content recovered from biological material, is increasing with significant advancements in paleogenomic technologies resulting in the characterization of over 1,700 ancient microbial genomes, partial genomes, and metagenomes. However, to date, most studies focused on aligning sequences to modern reference genomes, limiting discoveries to known taxa and their close relatives, and introducing a reference bias, since the genetic variability present in the modern genome is different from the one present in the ancient genome. A way to overcome this issue would be to perform a de novo assembly of metagenomes, resulting in a set of genomes of all the species that are present in the sample. The optimal procedure to do this involves high-quality genomic data consisting of long contiguous sequences, which can be challenging to obtain using current methods from fragmented ancient DNA (aDNA). Several pipelines have been created and applied in different studies, however, to date, there is not a golden standard to follow when analyzing ancient metagenomic data. In this study, we tested different de novo assembly pipelines in both simulated aDNA metagenomes and actual ancient dental calculus data. We compared the level of completeness and contamination of the different generated assemblies to identify the pipeline that better reconstructs the true ancient bacterial genomes. This pipeline can be used in future studies as a golden standard in the reconstruction of ancient metagenome-assembled genomes (MAGs), removing the reference bias that most studies introduce when mapping ancient genome bacteria against a modern genome reference.

POSTER

# Functional redundancy in microbial ecosystems

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A healthy ecosystem is generally characterized by the stable performance of essential ecological functions, which are typically supported redundantly by multiple species. Although the concept of functional redundancy is well-established in macroecology, estimating redundancy in microbial communities remains challenging and has thus been only partially explored. In this study, we aim to quantify the redundancy within the complex community of microorganisms in the gastrointestinal tract to predict the risk of functional loss in the human gut microbiome, thereby assessing the potential risk of dysbiosis. We specified a diversity-based redundancy index and applied it to metagenomics samples from obese patients before and after bariatric surgery. We observed a general increase in functional redundancy 12 months after bariatric surgery for nearly all functions showing significant differences. Our findings support the assumption that a high level of redundancy is typically associated with a healthier microbiome, thereby highlighting the importance of functional redundancy as a key biodiversity metric for microbiome stability and resilience.

POSTER

# Phylogeography and population genetics of the endemic Italian water voles, *Arvicola italicus*

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The genus Arvicola has a wide palearctic distribution and presents two ecological forms - terrestrial and aquatic - characterizing the genus. In Europe two species are present: the most widespread, *A. amphibius* and *A. italicus*, endemic to Italy. The aim of the study was to describe the genetic structure and variability in *A. italicus* and the genetic identity of populations in northeast Italy, an area of biogeographic importance as contact zone for small mammal species. Thirty-six Italian water voles, displaced in population from south to north of Italy, were analyzed by two mitochondrial markers and 8 autosomal microsatellite loci. The phylogenetic analysis on the genus confirmed the presence of major groups mostly corresponding to the species and clades already described. About the Italian specimens, the individuals from one population in northeastern Italy resulted included in *A. amphibius* revealing the presence of this species in Italy and, in consequence, the possible presence of a putative contact zone between *A. italicus* and *A. amphibius* in the northeast. The phylogeny includes a second population from northeast Italy within *A. amphibius*, but in basal position and poorly supported. The values of divergence of this lineage from *A. italicus* and *A. amphibius* lie in between intraspecific and interspecific divergence. Conversely, microsatellite data support a clear distinction between the two species and includes the ambiguous mitochondrial lineage in *A. italicus*. Furthermore, this northeastern population shows admixture of nuclear loci with the southcentral *A. italicus* populations, supporting the attribution of these specimens to *A. italicus*. This pattern could suggest that *A. italicus* in northeast Italy retained ancestral haplotype and undergone a long period of isolation respect to the southern populations, without genetic exchange until recent times. Within *A. italicus*, the admixture shows northern population as the most differentiated showing limited admixture with the most diverse southcentral. The intraspecific diversity decreases from south to north as expected for an endemic small mammal in Italy.

POSTER

# Elucidating bear eco-evolutionary dynamics using ecological and morphological evolutionary landscapes

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Bears (Ursidae, Carnivora) are considered keystone species in several ecosystems, besides their potential impact on early human migrations and habitat use. Fitness variations linked to phenotypic and/or ecological adaptations are rendered using evolutionary landscapes meant to show differences in selective advantage of specific traits. Bears, thanks to their phenotypic, dietary, and behavioral adaptations, represent an ideal case study for elucidating existing eco-evolutionary optima and potential trade-offs in large-sized mammals. Here we investigate the conformation of morphological and ecological evolutionary landscapes within this clade. 3D geometric morphometrics is applied to bear crania belonging to 23 living and extinct species for collecting shape data. Ecological variable scores are obtained relying on species occurrences from online databases (PaleobioDB, GBIF) and pre-existing literature, combined with paleoclimatic data (PALEO-PGEM). Our results suggest that strongest morphological and ecological adaptive peaks are mainly occupied by extinct derived morphotypes (i.e., short-faced bears). Both morphological and ecological landscapes show that many living species (e.g., genus Ursus) occupy a weakest selective optimum, whereas extant specialists (e.g., polar bears, pandas) are far from the strongest peaks. These evidences suggest that most of living bears are characterized by non-extreme morphologies and ecological niches that probably give them higher ecomorphological flexibility than their fossil counterparts, which occupied stronger selective peaks at the expenses of an increased extinction risk (e.g., potential occurrence of ratchet-like mechanisms in short-faced bear evolution).

POSTER

<u>Eligible for Best Poster Prize</u>

# Evidence of convergent evolution in the nuclear and mitochondrial OXPHOS genes across Squamata deep lineages

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The oxidative phosphorylation system (OXPHOS) is composed of five complexes, with subunits encoded by both the mitochondrial genome (mtOXPHOS) and the nuclear genome (nucOXPHOS). mtOXPHOS and nucOXPHOS subunits tightly interact; indeed, signs of coevolution between these two sets of genes have been observed in Metazoa. Squamata is particularly intriguing from a mitochondrial point of view: the phylogenetic signal inferred from mitochondrial markers is radically discordant compared to nuclear markers. According to mitochondrial topology, lizards of the family Agamidae (Acrodonta) are in sister relationship with the crown-group of snakes (Serpentes), rejecting the monophyly of Iguania (Acrodonta + Pleurodonta), which is supported by most phylogenetic studies on Squamata. It has been proposed that the convergence between the early lineages of snakes and agamids is due to a common selective pressure on OXPHOS. In this study, we aim to investigate whether convergent evolution between these two lineages (i.e., Serpentes and Agamidae) is also detectable in nucOXPHOS. We annotated 76 nuclear OXPHOS genes from 56 Squamata genomes encompassing 24 families, along with the 13 mitochondrial OXPHOS genes. Phylogenetic trees were inferred from both datasets, and we evaluated signals of convergent evolution between Agamidae and Serpentes. The evolutionary rate correlation analysis revealed a clear coevolutionary signal between nucOXPHOS and mtOXPHOS, which is stronger when considering only subunits in close contact within the complexes. Moreover, some nucOXPHOS genes resulted under positive selection along the Serpentes and Acrodonta lineages, with clues of convergent substitutions observed between the two.