



# XIX ANNUAL MEETING OF THE PORTUGUESE ASSOCIATION FOR EVOLUTIONARY BIOLOGY

XIX ENBE 2023

18-19 Dezembro, Lisboa

Hosted by Instituto Superior de Agronomia & Faculdade de Ciências  
University of Lisbon, Portugal

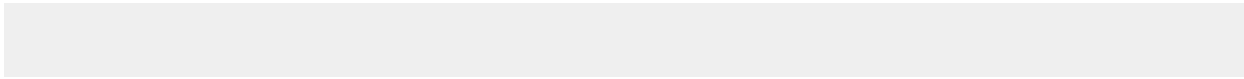
<https://enbe2023.rd.ciencias.ulisboa.pt>





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## ABOUT ENBE

The international meeting of Portuguese Association for Evolutionary Biology (ENBE) is an annual meeting that aims to bring together Evolutionary Biologists working in Portugal and abroad in order to promote scientific cohesion and excellence. This meeting is a forum for scientists of all academic levels (from master students to principal investigators), to present their work and discuss, fostering new ideas and collaborations. We invite researchers from all over the world to participate and submit their work.

XIX ENBE will be held in the **auditorium of the Instituto Superior de Agronomia / Universidade Lisboa (ISA/ULisboa)** on **December 18 and 19, 2023**.

Students (MSc, PhD) presenting their work are eligible for the [APBE awards](#) for the best oral and poster presentation.



## ORGANIZING AND SCIENTIFIC COMMITTEES

### **Association for Evolutionary Biology (APBE)**

ENBE is an annual meeting promoted by the Portuguese Association for Evolutionary Biology (“Associação Portuguesa de Biologia Evolutiva” – APBE) in collaboration with universities and research centres in Portugal.

### **XIX ENBE ORGANIZING COMMITTEE**

Chairman: Maria Romeiras (ISA, Universidade Lisboa)

Ana Ribeiro-Barros (ISA, Universidade Lisboa)

André Almeida (ISA, Universidade Lisboa)

Élio Sucena (IGC e FCUL, Universidade Lisboa)

Dora Batista (ISA, Universidade Lisboa)

Filipa Monteiro (ISA, Universidade Lisboa)

Inês Fragata (FCUL, Universidade Lisboa)

Sara Magalhães (FCUL, Universidade Lisboa)

Vítor Sousa (FCUL, Universidade Lisboa)

### **XIX ENBE SCIENTIFIC COMMITTEE**

Margarida Matos (FCUL, Universidade Lisboa)

Sofia Seabra (IHMT, Universidade Nova Lisboa)

Maria Romeiras (ISA, Universidade Lisboa)

Rui Faria (CIBIO, Universidade Porto)

Gareth Pearson (Universidade Algarve)

Ana Abecassis (IHMT, Universidade Nova Lisboa)

Rita Ponce (ESS/IPS)

Mónica Marques (CIIMAR, Universidade Porto)

# COLLABORATORS

## **INSTITUTIONAL COLLABORATORS**

ISA – Instituto Superior de Agronomia, Universidade de Lisboa

LEAF – Linking Landscape, Environment, Agriculture and Food—Research Center

TERRA - Associated Laboratory for the Sustainability of Land Use and Ecosystem Services

cE3c – Center for Ecology, Evolution and Environmental Changes

Change – Global Change And Sustainability Institute

Ciências – FCUL Faculdade de Ciências da Universidade de Lisboa

IHMT – Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa

UP – Universidade do Porto

## LOCATION

This year the XIX ENBE will be held in Lisbon – Instituto Superior de Agronomia (ISA/University of Lisbon), on 18-19 December 2023.

The Instituto Superior de Agronomia is located in Lisbon, in Tapada da Ajuda, between Monsanto and Alcântara (near Largo do Calvário and next to the 25 de Abril bridge).

There are four access gates with the following opening times:

- **Jau Gate (access for pedestrians, bicycles, cars with ID card):** 7 a.m. to midnight (summer time)
- **Monsanto Gate (access for pedestrians, bicycles, cars with ID card):** 7:30 a.m. – 10:30 a.m. and 4:30 p.m. – 7:30 p.m. (weekdays)
- **Polo da Ajuda Gate (access for pedestrians, bicycles):** 8 a.m. to 9 p.m. (summer time)  
Cars with ID card: 8h00 – 10h00 and 16h00 – 18h00 (weekdays)
- **Bridge Gate (pedestrian and bicycle access):** 8 a.m. to 9 p.m. (summer time)

### PUBLIC TRANSPORTS:



Place	BUS	Tramway
Largo do Calvário	712, 714, 727, 732, 738, 751, 756	E15
Rua Jau (next to the Jau Gate)	760	E18
Next to the Bridge Gate	724	
Next to the Monsanto Gate	724	
Rua Luís de Camões	720, 742	

# Na Tapada da Ajuda

XIX ENBE will be held at ISA Main Building (Photo left side) – Sala de Atos (Photo right side). Lunch on December 19th will be held at a private area of the ISA canteen.



# ACKNOWLEDGMENTS

## TECHNICAL SUPPORT

Ana Luísa Soares (ISA, Universidade Lisboa)

Luísa Valério (ISA, Universidade Lisboa)

Inês Diniz (ISA, Universidade Lisboa)

Silvia Catarino (FCUL, Universidade Lisboa)

Vanézia Rocha (ISA, Universidade Lisboa)

Ana Raquel Cunha (ISA, Universidade de Lisboa)

Carolina Branco (ISA, Universidade Lisboa)

Mário Rui (ISA, Universidade Lisboa)



# PROGRAM

## DAY 1

TIME	18-Dec
12:30	Registration
12:40	
12:50	
13:00	
13:10	
13:20	
13:30	
13:40	<b>Welcome:</b>
13:50	Vice-President of ISA Luís Goulão; Chair of ENBE2023 Maria Romeiras; President of APBE Sara Magalhães
14:00	Plenary 1:
14:10	
14:20	
14:30	
14:40	<b>Picão Osório:</b> The evolution of developmental genetic biases explains divergent evolutionary rates
14:50	<b>Martínez Monreal:</b> Phenotypic evolution of the marine gastropod <i>Littorina obtusata</i> across an environmental gradient at high latitudes
15:00	<b>Vishwakarma:</b> The Genetics of Expansions and Contractions across varied Life-History Traits
15:10	<b>Frazão:</b> Gut bacterial evolution during pregnancy
15:20	<b>Pinto:</b> Deconstructing the glomerular kidney: a case of evolutionary parallelism in teleosts
15:30	<b>Paulo:</b> Mechanisms of adaptation to oral infection in <i>D. melanogaster</i> upon experimental evolution
15:40	<b>Almeida:</b> The use of Proteomics and Metabolomics to understand adaptation to Seasonal Weight Loss in different sheep breeds
15:50	Discussion: Chair section Élio Sucena
16:00	
16:10	Coffee break / Poster Section
16:20	
16:30	
16:40	<b>Curto:</b> The diversification of <i>Micromeria</i> Benth. (Lamiaceae) in the Canary Islands reflects species syngameon dynamics
16:50	<b>Andrade:</b> Aspects of reproductive biology, pollinators, and taxonomy of <i>Musschia</i> Dumort. (Campanulaceae)
17:00	<b>Galhofa:</b> A Genotyping-by-Sequencing Approach Brings New Insights into the Population Structure and Local Adaptation of Western Mediterranean Oaks
17:10	<b>Batista:</b> Evolutionary dynamics in an apparently asexual crop fungal pathogen: the case of coffee rust
17:20	<b>Carvalho:</b> Diploid Nuclei Occur throughout the Life Cycles of Pucciniales Fungi
17:30	<b>Marques:</b> The return of the monsters: natural hybridization as a source of novelties in Neotropical orchids
17:40	<b>Monteiro:</b> A pantropical population genetics study on cashew crop: uncovering genetic diversity and agrobiodiversity hotspots
17:50	<b>Ribeiro-Barros:</b> Plant-Microbe Interactions: Friends and Foes
18:00	<b>Romeiras:</b> Macaronesian Islands as "natural laboratories" to study patterns of evolution and biogeography
18:10	Discussion: Chair section Dora Batista
18:20	

## DAY 2 - MORNING

TIME	19-Dec
09:00	
09:10	
09:20	<b>Plenary 2:</b> <b>Marta Montserrat Larrosa:</b> "Food web engineering: Ecology and evolution to improve biological pest control"
09:30	
09:40	<b>Grandela:</b> Mating behaviour and fertility of a monandrous insect under thermal stress
09:50	<b>Ferreira:</b> The impact of environmental heterogeneity on the genomics of adaptation to cadmium
10:00	<b>Henriques:</b> Phenotypic and genetic variation for competitive ability and their impact on long-term interactions between spiders mites
10:10	<b>Costa:</b> Surviving the heat: How multiple mating rescues offspring sex ratio in a haplodiploid exposed to developmental heat stress
10:20	<b>Kozak:</b> Experimental evolution reveals a plastic response to ecological and physiological costs of suppressing plant defences
10:30	<b>King:</b> How does ecological stability affect evolution?
10:40	
10:50	<b>Discussion:</b> Chair Section Margarida Matos
11:00	
11:10	<b>Coffee break / Poster Section</b>
11:20	<b>Lobo:</b> The population bottleneck of the Iberian wolf impacted genetic diversity but not admixture with domestic dogs: a temporal genomic approach
11:30	<b>Rocha:</b> Multiple mitochondrial introgression events from an ancient lineage of Rüppell's fox ( <i>Vulpes rueppelli</i> ) into red fox ( <i>Vulpes vulpes</i> )
11:40	<b>Ribeiro:</b> The value of captive wildlife in the conservation of species: Exploring wild origins and genetic diversity in the iconic sable antelope
11:50	<b>Pinho:</b> Table for two: diet composition differences of allopatric and sympatric populations of island geckos
12:00	<b>Bernardo:</b> Signatures of adaptation at the mitochondrial level in endemic freshwater fish species from contrasting environments
12:10	
12:20	<b>Discussion:</b> Chair section Rui Faria
12:30	
12:40	
12:50	
13:00	
13:10	<b>Conference Lunch</b>
13:20	
13:30	
13:40	
13:50	

## DAY 2 – AFTERNOON

TIME	19-Dec
14:00	
14:10	
14:20	<b>Plenary 3:</b> <b>Susana Coelho:</b> '(A)sex in the rockpools: the private life of algae'
14:30	
14:40	<b>Arbore:</b> The molecular bases of red-to-yellow color variation in parrots
14:50	<b>Figueiredo:</b> Abandoned gold mine reveals new mysterious nuggets: Redescription of three fossil whales from the Miocene of Portugal
15:00	<b>Moreno:</b> Phenotypic plasticity in response to climate change in Iberian chubs: insights from proteomics and phosphoproteomics
15:10	<b>Mendes:</b> Contrasting outcomes of hybridization in Iberian chubs revealed through whole genome data
15:20	<b>Choechaipaisarn:</b> Density-dependent dispersal promotes female-biased sex allocation and reduces sex-ratio conflict in viscous populations
15:30	<b>Blanckaert:</b> Interactions between mechanisms of reproductive isolation
15:40	<b>Lima:</b> Human migrations and gene flow with local dogs shaped the genomic ancestry of livestock guarding dogs across Eurasia
15:50	
16:00	<b>Discussion:</b> Chair section Sofia Seabra
16:10	
16:20	<b>Coffee break / Poster Section</b>
16:30	
16:40	
16:50	<b>Students awards &amp; Closing Session</b>
17:00	
17:10	
17:20	
17:30	<b>APBE ASSEMBLY</b>
17:40	
17:50	

## PLENARY SPEAKERS

### Brian Lazzaro



**Department of Ecology and Evolution, Cornell University, USA**

Brian's research focuses on the evolutionary and functional genetics of insect-pathogen interactions. More specifically, he has been working on identifying host and microbe genetic and/or environmental factors that shape the outcome of infections.

### Marta Montserrat Larrosa



**Institute for Mediterranean and Subtropical Horticulture La Mayora, Spain**

Marta's combines ecology and evolutionary biology to improve the management of agro-ecosystems exposed to the negative effects of warming. More specifically she studies how temperature and food community structure affect interactions between species with the ultimate goal to help pest control management.

### Susana Coelho



**Max Planck Institute for Biology, Tubingen, Germany**

Susana's research focuses on understanding the origin and evolution of different sexual systems, as well as understanding how developmental patterns are regulated and how they evolve. More specifically she studies how sexual chromosomes evolve and how are major transitions in sexual systems regulated and evolve using the brown algae study system.

# ABSTRACTS OF PLENARY TALKS

## DAY 1

### **Endocrine signaling and physiological constraint drive a reproduction-immunity tradeoff in *Drosophila***

**Brian Lazzarro**

Department of Ecology and Evolution, Cornell University, USA

Life history tradeoffs between reproductive output and immune performance are commonly observed throughout the animal kingdom but are rarely understood mechanistically. In the insect *Drosophila melanogaster*, reproductive investment reduces resistance to bacterial infection. We have found that the reduction in immunity can be traced to the cost of producing eggs, and that mating-induced changes in hormone balance regulate the differential investment in fecundity versus immunity. However, rather than arising through an adaptive shift in allocation of energetic resources from one process to the other, the tradeoff seems to originate in physiological constraints and overburdening of the fat body tissue. We observe natural genetic polymorphism for the magnitude of post-mating immune suppression but it is largely uncorrelated with reproductive output, potentially limiting the opportunity for evolutionary optimization. Thus, while reproduction and immune performance are observed to trade off in *D. melanogaster* as would be expected under classical life history theory, the mechanism and evolutionary potential may not conform to classical expectations.

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## DAY 2

### Food web engineering: Ecology and evolution to improve biological pest control

Marta Montserrat Larrosa

Institute for Mediterranean and Subtropical Horticulture La Mayora, Spain

The 2030 UUNN agenda for sustainable development demands to assure food supply via the implementation of sustainable and resilient agricultural practices that strengthen agricultural systems capacity for adaptation to environmental change. A key component to achieve resilience and sustainability in agricultural systems is biological pest control (BPC), that is, the use of biological control agents (BCAs) to control the population of pests. The rate of failure of BPC, however, is estimated to be as high as 68 %, and we think that biotic (e.g. presence of other species that also interact with the BCAs) and abiotic (e.g. warming) stressors negatively affecting the performance of BCAs in the field are an important cause of these failures.

In this seminar I will, firstly, explain the rationale behind food web engineering (FWE), which seeks to combine knowledge in evolutionary biology with that of food web theory to engineer webs that are efficient at keeping crop pests at bay, via exploiting the inherent intraspecific variability in the populations of BCAs to improve their performance when they are exposed to hostile environments. Secondly, I will present the results of experimental evolution and artificial selection carried out using the predatory mite *Amblyseius swirskii*, one of the most frequently used BCA at present, within the context of the research in FWE carried out in our lab.

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### (A)sex in the rockpools: the private life of algae

Susana Coelho

Max Planck Institute for Biology, Tubingen, Germany

Sexual reproduction is an ancient and conserved feature of life on earth, but the mechanisms that determine the sex of an individual are mesmerizingly diverse and have had rapid turnover rates during evolution. What drives sex chromosome evolution? What are the mechanisms underlying switches between sex determination systems? Are all sex chromosomes equal in terms of origin and evolutionary trajectories? The answers are complex but the ongoing genomic revolution and the use of alternative model organisms is shedding new light on sex chromosome diversity and evolution. I will describe how brown algae, which have been evolving independently from animal and plants for more than a billion years, are contributing to this dynamic field of research.

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# ABSTRACTS OF THE ORAL PRESENTATIONS

## The evolution of developmental genetic biases explains divergent evolutionary rates

Picao-Osorio, J (1,2); Bouleau, C (3); Gonzalez de la Rosa, PM (4); Stevens, L (4); Fekonja, N (1); Blaxter, M (4); Braendle, C (3); Félix, M-A (1)

(1) IBENS - Institut de Biologie de l'École Normale Supérieure, CNRS, INSERM, ENS, PSL, Paris, France; (2) CE3C - Centre for Ecology, Evolution and Environmental Changes, Department of Animal Biology, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; (3) IBV - Institut de Biologie Valrose, Université Côte d'Azur, CNRS, INSERM, Nice, France; (4) Tree of Life, Wellcome Sanger Institute, Cambridge, UK.

Understanding the mechanisms that generate phenotypic variation and their impact on the course of evolution is a central challenge in modern biology. The process of development may limit or bias the phenotypic spectrum obtained after random mutation, deemed developmental bias. Therefore, it is essential to understand these biases in order to reveal which phenotypes can be explored and selected in the evolutionary process. Despite this, it is still poorly understood whether developmental genetic biases in the production of phenotypic variation influence evolutionary rates. Here, we address this problem by quantifying the relationship between mutation and wild phenotypic variation within and among nematode species using a quantitative genetics approach. We use the homologous cellular framework of the six vulval precursor cells (VPC), named P3.p to P8.p, in two clades of nematodes that have divergent evolutionary trajectories of cell fate variation. First, we quantified the evolutionary rates of VPC cell fate variation in two nematode genera that diverged around 80 million years ago, *Caenorhabditis* and *Oscheius*, using phylogenetic mixed models. We show that among *Caenorhabditis* species, P3.p cell fate variation displays the highest evolutionary rate, while in *Oscheius* species it is P4.p. Second, we demonstrate that these patterns of macro-evolutionary variation are akin to the micro-evolutionary variation estimated within species of both genera. That is, P3.p and P4.p vary extensively within *Caenorhabditis* and *Oscheius* species, respectively. Third, to see to what extent these divergent evolutionary rates were due to the evolution of developmental bias, we generated eight panels of random mutant lines in wild isolates of both genera to quantify the mutability (i.e., mutational variance) of VPC fates across micro- and macro-evolutionary scales. Then, we compared these mutational variances with natural genetic variation within and across species of both genera. Our phenotypic analysis of vulva cell fates on over 85,000 nematodes shows a strong alignment of the axes of variation upon random mutation with those of wild variation within each species and genus, but contrasting between genera. When represented in a simplified two-dimensional phenotypic space, the direction of mutational and natural variation within and among *Caenorhabditis* species is along the P3.p axis, and along the P4.p axis in *Oscheius* species. This shows that the evolution of developmental biases predicts more than 80 million years of divergent phenotypic evolutionary rates. Altogether, we show how the evolution of the variational properties of cell fate decisions and their underlying developmental genetic mechanisms explain the evolution of evolutionary rates.

Presented by João Picão Osório ([joao.picao.osorio@fc.ul.pt](mailto:joao.picao.osorio@fc.ul.pt))

## Phenotypic evolution of the marine gastropod *Littorina obtusata* across an environmental gradient at high latitudes

Pires, A. (1,2); Monreal, I. (1); Gaio R. (2); Pálsson S. (3); Raeymaekers J. (4); Westram A. (4); Faria R. (1)

(1) CIBIO - Centro de Investigacao em Biodiversidade e Recursos Genéticos, Universidade do Porto, Porto, Portugal; (2) FCUP - Faculty of Science of the University of Porto, Porto, Portugal; (3) Department of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland; (4) FBA - Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

Species of the genus *Littorina* are among the most extensively studied marine gastropods. Its potential as model for studies in evolutionary ecology lies in their distribution across the intertidal zone (where organisms experience strong selective pressures), the occurrence of multiple intraspecific polymorphisms in shell morphology associated with different habitats (often called ecotypes) that likely evolved in parallel across its range, and interspecific variation in life history traits. In *Littorina obtusata*, the focal taxon of this study, two varieties have been described: *palliata* and *retusa* (with higher and lower spire, respectively). In Europe, the former is confined to the shores northern of the Lofoten Islands (Norway) and in the north and east of Iceland, while the latter variety is common elsewhere. The European green crab (*Carcinus maenas*) together with temperature have been suggested to drive this polymorphism in the Western Atlantic, a detailed characterization of the phenotypes as well as the evolutionary mechanisms involved remains to be done in European shores. In order to fill this gap, a thorough characterization of shell phenotypes from five Norwegian *L. obtusata* populations was implemented to quantify the main phenotypic differences across the latitudinal gradient encompassing the geographic transition between the two varieties to provide insights about the main forces and environmental/biotic factors driving their evolution. The analysis of total weight, shell thickness, total height, spire/total height, spire angle, and shell strength revealed a trend of increasing average values from northern to southern populations, except for spire/total height, in agreement to previously reported for *palliata* and *retusa* varieties. Air and sea water temperature, salinity, and the presence of crabs all vary in the same direction, making it difficult to pinpoint the main driving factor of this polymorphism. The populations located in intermediate latitudes of our transect, showed intermediate values or clustered either with the northern vs or southern populations, depending on the trait. This suggests that the transition between the two varieties could be northern than previously described, highlighting the potential of this regionsystem to study how global warming affects the ecoevolutionary dynamics of biodiversity in marine coastal areas.

Presented by Isabel Martínez Monreal ([isa.nord20@gmail.com](mailto:isa.nord20@gmail.com))

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## The Genetics of Expansions and Contractions across varied Life-History Traits

Vishwakarma, R (1); Sgarlata, GM(1); Soriano-Paños, D(1); Tournebize, R(1,2); Maié, T(1,3); Rasteiro, R (1,4); Chikhi, L(1,2)

IGC - Instituto Gulbenkian de Ciência, Oeiras, Portugal; (2) EDB - Laboratoire Evolution et Diversité Biologique, UMR 5174, CNRS, IRD, Université Toulouse Paul Sabatier, 31062 Toulouse, France; (3) ICG - Institute for Computational Genomics, RWTH Aachen University; (4) SBS - School of Biological Sciences, University of Bristol, Bristol BS8 1TL, UK

Species ranges are dynamic, changing over their natural history due to various factors such as climate change and, more recently, human activities. These changes encompass a range of spatial processes, including expansion, contraction, shifts, or combinations thereof, resulting from underlying intrinsic (species related) and extrinsic factors. While previous studies extensively explored the genetic effects of these spatial processes, they rarely incorporate life-history traits. Thus, there remains a gap in our understanding regarding the variations in genetic diversity patterns among species with distinct life-history traits experiencing similar habitat change scenarios. In this study, we utilised spatial simulations and a panmictic population model to investigate the temporal dynamics of genetic diversity within refugium populations experiencing a range expansion followed by a contraction, while considering various contraction speeds and life-history traits. We identified three temporal dynamics of genetic diversity in the refugium population duration contraction phase: i) a decrease, ii) an initial increase followed by a decrease or iii) an increase. These different temporal dynamics arise due to the fact that populations deviate from the genetic diversity expected for their population size and habitat. We also observe two patterns of the effect of contraction speed on the genetic diversity at the end of contraction. These two patterns are: as the contraction time is increased (or contraction speed is slowed), (i) genetic diversity sampled at the end of contraction can monotonically decrease, or, (ii) genetic diversity can initially increase and then decrease, following a non-monotonic pattern.

Presented by Ravi Vishwakarma (ravivishwakarma1996@gmail.com) *Eligible for best talk award*

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## Gut bacterial evolution during pregnancy

Bom, J (1); Seixas, E (1); Frazão, N (1)

(1) IGC - Instituto Gulbenkian de Ciência, Oeiras, Portugal

The dynamics of microbial communities in the gut undergo intricate changes during pregnancy, potentially affecting overall host-microbe interactions. In this study, our objective was to elucidate the molecular evolution of *Escherichia coli* (*E. coli*) within the gut of pregnant mice. Employing a gut colonization approach, we isolated *E. coli* populations from the gut of both pregnant and non-pregnant mice, subsequently subjecting them to whole-genome sequencing. Our analysis revealed that the rate of *E. coli* evolution is identical in pregnant and non-pregnant animals, with both *E. coli* populations showing signs of adaptation ( $dN/dS > 1$ ). The number of *E. coli*-specific mutations was more variable among pregnant animals, in line with the dysbiotic gut environment during pregnancy, attributed to higher levels of inflammation. A unique adaptive mutation emerged exclusively within *E. coli* populations isolated from pregnant mice. This intergenic mutation was associated with the import of cysteine, a crucial amino acid with potential bacterial cytotoxic properties. Given the anticipated increase in cysteine availability in the gut due to elevated food intake during pregnancy, we hypothesize that this mutation confers a selective advantage by mitigating excessive cysteine uptake. Such an adaptive mutation could play a pivotal role in safeguarding *E. coli* from cysteine-induced toxicity during pregnancy and subsequent transmission to newborns. This study provides novel insight into the adaptive mechanisms of *E. coli* populations in response to the distinct physiological conditions imposed by pregnancy. The identification of a pregnancy-associated mutational target related to cysteine transport sheds light on the intricate interplay between host physiology, diet, and microbial evolution within the gut ecosystem. Further research on the functional implications of this mutation will deepen our understanding of the microbial strategies that contribute to the stability and resilience of the gut microbiota during pregnancy and are transmitted to the newborn's gut.

Presented by Nelson Frazão ([nfrazao@igc.gulbenkian.pt](mailto:nfrazao@igc.gulbenkian.pt))

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## Deconstructing the glomerular kidney: a case of evolutionary parallelism in teleosts

Pinto, B (1,2); Machado AM (1,2); Gomes-dos-Santos A (1,2); Froufe E (2); Cordeiro JM (2); Barros-Garcia D (2); Kolbadinezhad SM (2,3); Fonseca E (2); Andrade JP (4); Palma J (4); Ruivo R (2); Monteiro (5,6); Wilson JM (2,7); Castro LFC (1,2)

(1) FCUP - Faculdade de Ciências da Universidade do Porto, Porto, Portugal (2) CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal (3) CFRC - Coldwater Fisheries Research Center; IFSRI - Iranian Fisheries Sciences Research Institute; Agricultural Research, Education and Extension Organization, Tonekabon, Iran (4) CCMAR - Centro de Ciências do Mar, Faro, Portugal (5) CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal (6) IUCN SSC Seahorse, Pipefish and Seadragon Specialist Group, Vancouver, Canada (7) Department of Biology, Wilfrid Laurier University, Waterloo, Canada

The nephron, the fundamental unit of the kidney, evolved in the ancestor of the Bilateria from the elaboration of an ultrafiltration-based organ to produce urine. The functional segmentation of the nephron is present in most extant vertebrate lineages, with the presence of a filtering element, the glomeruli—a network of capillaries contained within the Bowman’s capsule. This filtration system is based on a specific cell type, the podocyte, which displays slit diaphragms through which a regulated filtration takes place ultimately forming the urine. Strikingly, not all vertebrate species display a glomerular kidney structure. Previous studies have shown that seven marine teleost lineages have been classified as “aglomerular” on the basis of morphology, including the iconic *Syngnathidae* (seahorses, seadragons and pipefishes), *Notothenioidei* (Antarctic fishes) and *Batrachoididae* (toadfishes). The molecular foundations for the independent emergence of the aglomerular phenotype are unknown. By means of comparative genomics and transcriptomics, we deduce that a set of structural proteins, including nephrin and podocin, as well as specific glucose transporter have been subject to gene deletion in these lineages. To our knowledge this parallel evolutionary condition was previously undescribed in vertebrate species. To further inspect the physiological implications related to the erosion of this set of genes, which are vital precursors to the formation of the glomerulus and its normal functionality, a comparative transcriptomics analysis was performed between *Syngnathidae* and reference species (*Thunnus thynnus* and *Lates calcarifer*). Three new *Syngnathidae* kidney projects were generated and, alongside the already publicly available datasets, subjected to gene expression filtering and enrichment analyses against the gene repertoire background of *Danio Rerio*. Our findings revealed a pattern that is consistent with a major renal adjustment, from a typical filtration device to a predominantly excretory system. Finally, we propose that aglomerulism evolved independently in various teleost lineages, including *Syngnathidae* and *Batrachoididae*, as an energy saving adaptation, since it does not require high rates of energy-intensive water reabsorption. For species like the toadfish, which are primarily benthic ambush hunters, this comes as a major advantage to cope with their sedentary lifestyle. Regarding *Syngnathidae*, which are also ambush hunters famously known for their low energy-based diet and inefficient utilization of fuel from food, the loss of an energetically costly process is paramount. For *Nototheniidae*, a possible explanation for this phenotype might be related to antifreeze mechanisms. Polar fishes, while ingesting polar seawater, are vulnerable to the ingestion of ice crystals that can cause internal freezing and consequent death. Because of this, they have evolved to rely on APs (Antifreeze proteins) that inhibit the growth of the ingested ice that enters their blood or other body fluids. These proteins lower the fish’s freezing temperature, making them less susceptible to death by internal freezing. Studies which tested the urine composition of polar fishes concluded that there is a substantial amount of APs present in the urine. According to past

research focused on the morphology of *Nototheniidae*, species that inhabit regions in which the water temperature can reach negative values, and are consequently equipped with antifreeze glycopeptides, display an aglomerular phenotype. A plausible theory for the aglomerular emergence in subzero marine teleost species might be that it results in a urinary conservation of these APs, which would otherwise not be maintained in the organism. Together our results highlight the essential role of secondary gene loss in generating phenotypic diversity.

Presented by Bernardo Pinto ([bernardo.mlj.pinto@gmail.com](mailto:bernardo.mlj.pinto@gmail.com)) *Eligible for best talk award*

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## Mechanisms of adaptation to oral infection in *D. melanogaster* upon experimental evolution

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In natural environments, metazoan hosts are constantly enduring the action of pathogens. These interactions constitute strong evolutionary motors that have driven the emergence of the immune responses. As an animal model of reference, *Drosophila*'s immune response has been well characterized. However, most studies have relied on inbred lines, kept under laboratory conditions, with relatively weak ecological and evolutionary relevance. However, recent work has begun to study *Drosophila* immunity encompassing using natural pathogens in populations with abundant genetic variation, including previous work in the lab that established experimentally evolved populations of *D. melanogaster* under several pathogen challenges. In one such regime, an outbred population of *D. melanogaster* was selected against oral infection (BactOral) with a natural pathogen, *Pseudomonas entomophila*, acquiring after 6 generations of selection, a significantly increased survival upon infection, compared to the Control regime. To uncover the mechanistic bases of the rapid evolutionary trajectory of BactOral we performed a multi-level phenomenological characterization, alongside transcriptomic profiling. We tested several phenotypes relevant against oral bacterial infections, including feeding and bacterial defecation rates, bacterial load dynamics, and AMP expression levels. We concluded that adaptation in BactOral relied on a combination of higher resistance and possibly stronger disease tolerance. We conducted RNA-Seq on whole-bodies and on guts at 4 timepoints, revealing differences between populations during both infection progression and resolution. These results agreed with the previous observations of increased resistance of BactOral, at the gut level. Additionally, at the whole-organism level, BactOral exhibits an earlier downregulation of immune effectors that correlates to its higher disease tolerance. Finally, using conditional RNAi, we functionally validated the role of the candidate genes Drosomycin-like 2 and Lactate dehydrogenase in the immune response of *Drosophila*, possibly in the control of bacterial loads and localized gut immunity. In sum, this work systematically addresses several layers of the immune response, gaining a deeper understanding of how immunity can be shaped by evolution. Additionally, it highlights that resistance and disease tolerance can evolve in parallel, synergizing or additively contributing to higher fitness.

Presented by Tânia Paulo ([tania.f.paulo@gmail.com](mailto:tania.f.paulo@gmail.com))

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## The use of Proteomics and Metabolomics to understand adaptation to Seasonal Weight Loss in different sheep breeds

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Seasonal weight loss (SWL) is a pressing issue in animal production. Animals selected in SWL prone areas are well adapted to SWL. Understanding the molecular mechanisms of SWL adaptation is of high importance in animal selection. We studied the effect of SWL in meat-producing sheep. In this presentation, a retrospective of the work conducted over the last 20 years is presented, highlighting major findings within this research line. The objective of this research was to characterize the production traits (growth, carcass and meat quality) and the differential protein expression in the skeletal muscle of three different breeds of sheep: the Australian Merino, the Dorper and the Damara, each showing different levels of tolerance to weight loss (low, medium and high, respectively). Per breed, two experimental groups were established using 6 month-old ram lambs (n=12). One group was labelled “Growth” and the other labelled “Restricted.” After forty-two days of dietary treatment, all animals were euthanized in a commercial abattoir and carcass and meat traits determined. Muscle samples were then taken and Proteomics profiles determined using two dimensional electrophoresis coupled to mass spectrometry (MALDI-TOF/TOF), as well as label free proteomics. Results point out to important differences between the merino and the South African breeds (Dorper and Damara) regarding carcass and meat quality traits like carcass yield and meat color (darker in the Damara breed). Regarding the 2D Proteomics analysis, results also reveal relevant differences between breeds with several proteins are suggested as putative biomarkers of tolerance to weight loss: Desmin, Troponin T, Phosphoglucomutase and the Histidine Triad nucleotide-binding protein 1. Moreover, we suggest as related to SWL tolerance: S100-A10 Serpin A3-5-like and Catalase. From the label free proteomics experiment, we observe that the more vulnerable to SWL a breed is, the more differential abundance proteins we find. Protein binding was the most frequently altered molecular function identified. We finally suggest 6 putative markers for restricted nutritional conditions independently of breed: ferritin heavy-chain; immunoglobulin V lambda chain; transgelin; fatty acid synthase; glutathione S-transferase A2; dihydrodiol dehydrogenase 3-like. This information is finally of relevance to and of possible use in selection programs aiming towards ruminant animal production in regions prone to droughts and weight loss. Finally, we studied also the effect of SWL on the wool proteome of Australian Merino lambs. A proteomic approach has been applied to study changes in fiber structure and protein composition in wool from merino sheep subjected to experimentally induced weight loss. Results indicate that there is a significant reduction in the fiber diameter of wool from the animals on a restricted diet over a 42-day period. At the same time, significant increases in the expression of the high sulfur protein KAP13.1 and proteins from the high glycine-tyrosine protein KAP6 family in the wools from the animals on the restricted diet were also detected. Such findings have strong implications for the wool industry that favors finer wool.

Presented by André Almeida ([aalmeida@isa.ulisboa.pt](mailto:aalmeida@isa.ulisboa.pt))

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## The diversification of *Micromeria* Benth. (Lamiaceae) in the Canary Islands reflects species syngameon dynamics.

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Hybridization can promote adaptive radiation by increasing standing variation and consequently the ability of an organism to adapt to new conditions. In oceanic islands, this mechanism may be valuable in counteracting founder effects through the admixture of lineages arriving to an archipelago by independent colonization events. The resulting species would evolve connected by geneflow in what is known as a syngameon. In archipelagos like the Canary Islands that are close to the mainland and the islands are relatively close to each other, multiple colonization events are expected to be frequent, so the formation of syngameons is expected to be an important process shaping diversity. We have been testing this hypothesis using the genus *Micromeria* (Lamiaceae) in the Canary Islands. Microsatellite genotyping recovered higher genetic diversity in recently colonized islands evidencing a mechanism contradicting founder effect. The same dataset supports the existence of intraspecific gene-flow, which was later confirmed using RAD-sequencing. These results support the idea that the formation of syngameons in *Micromeria* played an important role in the distribution of diversity during the diversification of this genus in the archipelago. So far, phylogenetic analyses have been based on nuclear variation of only a few individuals per species causing difficulties to infer the extent of gene flow between species. Additionally, microsatellite markers are not ideal to evaluate differences in genetic diversity across species. In the present study, we have overcome such limitations by using 6000 bp of chloroplast genome and 23 nuclear intron loci across several populations per species of *Micromeria* to assess phylogenetic and genetic diversity patterns that might indicate the establishment of species syngameons. Both gene- and species tree approaches were implemented using IQ-Tree, and the resulting patterns were compared among loci and marker systems. Differences in genetic diversity between species were estimated based on nucleotide and haplotype diversity. Intraspecific geneflow and admixture were estimated based on migration rates and haplotype sharing patterns across species. Trees combining information from all loci are congruent with species delimitation, which was not always the case for gene trees. Chloroplast haplotypes were shared among some of the highest divergent nuclear lineages in the archipelago. Nuclear loci showed higher genetic diversity and degree of intraspecific haplotype sharing in younger islands than in older ones, which was not the case for cpDNA variation, where no significant differences were found. Finally, intraspecific geneflow patterns were more complex in younger islands and usually involved the most widespread species. In summary the results are congruent with the establishment of syngameons during the diversification of *Micromeria* in the Canary Islands and support the idea that this is a process that minimizes founder effect. Syngameons seem more complex in the colonization front while more diluted in islands colonized a longer time ago. Widespread species seem to be the main contributors to the maintenance of intraspecific geneflow. Finally, the cytonuclear discordance found might be explained by the different evolutionary rates of chloroplast (slower) and nuclear introns (faster) reflecting different temporal scales.

Presented by Manuel Curto ([mcurto@cibio.up.pt](mailto:mcurto@cibio.up.pt))



## Aspects of reproductive biology, pollinators, and taxonomy of *Musschia* Dumort. (Campanulaceae)

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*Musschia* Dumort. (Campanulaceae) is an endemic genus of Madeira archipelago and its species are a clear example of adaptive radiation. *Musschia aurea* (L.f.) Dumort. is a perennial chamaephyte that lives in crevices of rocky cliffs of Mediterranean climate and in the inside of the valleys of Madeira island and Deserta Grande, *Musschia wollastonii* Lowe is a monocarpic microphanerophyte typical of rocky stream beds of the stink-laurel forest in Madeira, finally *Musschia isambertoii* M. Seq, R. Jardim, M. Silva & L. Carvalho is a monocarpic chamaephyte which grows in low altitude Mediterranean plant communities near to seashore, with poor soil development in Deserta Grande. *Musschia wollastonii* reproductive biology includes the presence of protandry, weak dichogamy, and a mechanism of secondary pollen presentation on the abaxial surface of the stigmatic lobes, simultaneously promoting outcrossing and selfing. Results show that *M. wollastonii* is self-compatible with an absent incompatibility system, with spontaneous selfing through delayed selfing. The flower visitors observed were insects from the Syrphidae, Nymphalidae, and Apidae families, all having larcenist behaviour, pollen thieving having an impact on reproductive success. Although pollinator role of both syrphids and butterflies cannot be ruled out. *Musschia aurea* (L.f.) Dumort. taxonomy has long been discussed. Two varieties were recorded by Ker Gawler (1815), the narrow leaf (*Campanula aurea*  $\beta$  *angustifolia*) found inside valleys and wide leaf (*Campanula aurea*  $\alpha$  *latifolia*). Later Dumortier (1823) described *Musschia angustifolia* Dumort. referring to the narrow leaf plants, but later authors do not recognize narrow-leaved plants as a taxonomic entity. Our results based on a biometric approach of leaf, flower, fruit, seed and pollen characters support the recognition of two taxonomically distinct groups of *Musschia aurea*: a larger group that comprises South and Northeast populations as *M. aurea* subsp. *aurea*; and a smaller group that includes populations from North and Northwest and altitudes above 600 m, as *M. aurea* subsp. *angustifolia*. We discuss the implications for conservation actions and ecosystem management of both the taxonomic clarification of *Musschia aurea* and the new data on the reproductive biology of *M. wollastonii*.

Presented by Tiago Andrade (tiagojga18@gmail.com) *Eligible for best talk award*

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## **A Genotyping-by-Sequencing approach brings new insights into the population structure and local adaptation of Western Mediterranean Oaks**

**Galhofa, D (1); Viegas, M (1); Sousa, F (1); Henriques, A (1); Sousa, P (1); Pina-Martins, F (1); Vanhove, M (2); Silva, A (3); Batista, D (4), Goulier-Lagadec, J (5); Galinat, F (6); Rzigui, T (7); Jalila, A (8); Ouadji, M(8); Forte, L (9); Cavallaro, V (9); Saulle, D (9); Dettori, S (10); Cillara, M (10); del Galdo, GG (11); Sciendrello, S (11); Picó, X (12); Marcer, A (13,14); Belkadi, B (15); Costa, A (16,17), Coelho, AC (1,18), Branquinho, C (3), Ingvarsson, PK (19), Knowles, LL (20), Paulo, OS (1)**

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The Mediterranean region has been described as a ‘climate change hotspot’, with increased temperatures and decreased precipitation expected to affect the region in the coming decades. Given the pace and intensity at which these changes are expected to happen, it becomes important to understand species capacity to respond to climate change. Species response to environmental change can happen through phenotypic plasticity, range shift, or genetic adaptation to their new conditions. In order to understand a species adaptive capacity to respond to climate change, it is important to disentangle how much of its genetic diversity is the result of population structure, and how much results from the action of natural selection. This work involved samples of Cork Oak (*Quercus suber*) and Holm Oak (*Quercus ilex* and *Quercus rotundifolia*), collected throughout the species range, with special attention given to the Western Mediterranean basin. Genotyping by Sequencing was employed to generate several genome wide Single Nucleotide Polymorphism datasets, which were used (i) to investigate population structure using several complementary approaches, and (ii) to detect evidence of local adaptation through a Landscape Genomics approach involving the detection of genetic-environmental associations with several bioclimatic variables. This work builds on previous analyses of Cork Oak SNP data, and is, to our knowledge, the first attempt to use genome-wide nuclear genetic markers to uncover the

existence of population structure and signatures of local adaptation in Holm oak. Our results reveal contrasting patterns of population structure and differentiation. Holm Oak shows a marked pattern of population structure and considerable differentiation, especially between *Q. rotundifolia* and *Q. ilex* samples, which brings support to the status of *Q. ilex* and *Q. rotundifolia* as two genetically distinct species. Cork Oak, on the other hand, shows much less pronounced population structuring, as reported in previous works based on nuclear genetic markers. Furthermore, a considerable degree of differentiation is observed between Iberian and Moroccan populations of *Q. rotundifolia*, which is not observed for *Q. suber*. Additionally, we uncover the relatively unstructured nature of the Iberian *Q. rotundifolia* and *Q. suber* populations. We also identified a considerable number of putative SNPs under selection in both species, showing association with multiple bioclimatic variables related to temperature and precipitation. Annotation of the genomic regions harboring these putative SNPs revealed several genes potentially associated with heat and water stress. In general, these results build on previous knowledge regarding the population structure of Cork Oak and bring new insights into the population structure of Holm Oak, contributing towards the clarification of its taxonomy, which up until this point has suffered from a lack of consistency. Regarding the detection of local adaptation, our results serve as a first step in understanding the capacity of Cork Oak and Holm Oak to respond to future climate change, opening the door to more complex analyses, such as genomic prediction of maladaptation, which may help identify areas of the species' distribution especially sensitive to climate change, and inform future management efforts towards the conservation of these species.

Presented by Davide Galhofa ([dmgalhofa@fc.ul.pt](mailto:dmgalhofa@fc.ul.pt))

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## Evolutionary dynamics in an apparently asexual crop fungal pathogen: the case of coffee rust

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Recurrent epidemics of fungal plant diseases constitute a growing worldwide problem and a major threat to global economy and food security. The fungal pathogen *Hemileia vastatrix* (Hv), causing the devastating coffee leaf rust (CLR) disease, has spread across the globe with heavy yield and revenue losses for Arabica coffee production. With the recent detection of CLR in Hawaii in 2020, the disease became endemic in all coffee producing regions of the world. Although the deployment of coffee resistant varieties has successfully contributed to control the disease, the highly adaptability of the fungus shaped by the dynamic system of host-pathogen co-evolution has been leading to the gradual breakdown of resistance in the field. Nowadays, more than 55 pathotypes (races) of Hv have been identified, but its virulence mechanisms are still poorly understood. Moreover, such ability to overcome resistance in coffee cultivars and evolve new races is puzzling for a pathogen that supposedly only utilizes clonal reproduction. Understanding the evolutionary complexity between Hv and its only known host, including determining how the pathogen evolves virulence so rapidly is crucial for disease management. In this communication, we will address the recent efforts in genomic research made by our group which started to unveil the population evolutionary history of this pathogen, the strong effect of local adaptation and breeding history, as well as footprints of introgression and recombination. In addition, some clues will be provided on possible mechanisms contributing to the generation of genetic diversity that might impact on virulence evolution. With the recent availability of a chromosome-level genome resource for Hv renewed prospects are considered for characterizing virulence loci and improving our knowledge on virulence divergence, envisioning the future development of candidate diagnostic markers associated to rust pathotypes.

Presented by Dora Batista ([dorabatista@isa.ulisboa.pt](mailto:dorabatista@isa.ulisboa.pt))

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## Diploid Nuclei Occur throughout the Life Cycles of Pucciniales Fungi

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Pucciniales (rust fungi) are biotrophic basidiomycetes that undergo the most complex life cycles in the fungal kingdom. This complexity has shaped the evolution of these organisms through host specialization and, probably, genome expansion. Rust fungi have a large genome size compared with the average genome size of other fungi. Fungal life cycles are characterised by prevalent haploid stages. In most cases, diploid nuclei occur only within a single cell, the basidium, after karyogamy of haploid nuclei. However, the haploid cycle is rapidly resumed since meiosis immediately follows. Rust fungi are also reported as obeying to this general rule. Conversely, our results challenge these well-established mycological principles. Using cytogenomic (flow cytometry and cell sorting) and cytogenetic (FISH with rDNA probes) approaches, we reported the occurrence of replicating haploid and diploid nuclei (i.e., 1C, 2C and a small proportion of 4C nuclei) in diverse life cycle stages (pycnial, aecial, uredinial, and telial) in a range of Pucciniales species analysed. These results suggest that the Pucciniales life cycle is distinct from any cycle known. This singularity appears to be transversal to the organisms in the Order and it does not occur in neighbouring taxa (Microbotryomycetes) nor on more distant taxa (Agaricomycetes and Ascomycota). This phenomenon may be a trait retained in rust fungi and lost in other lineages, as the early evolution of fungi may have been characterised by a diplontic life cycle. However, a biological explanation or function for the Pucciniales’ unique life cycle remains elusive.

Presented by Rita Carvalho (ritamdc.32@gmail.com) *Eligible for best talk award*

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## **The return of the monsters: natural hybridization as a source of novelties in Neotropical orchids**

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Biologists are often fascinated by evolutionary novelties among species such as novel traits, phenotypes, behaviors or new ecological niches. Yet, we rarely understand or can detect the context by which they were originated. Natural hybridization is a complex evolutionary process where such novelties can either be created or be blurred. For instance, introgression with one or both parental species can ease the progress of genetic swamping, and morphological homogenization. In other cases, introgressed genotypes and hybrids colonize new habitats, providing a pathway to the evolution of new lineages. Hybridization between species can also produce transgressive, novel, traits, and “monstrous” phenotypes well beyond the range of parental populations. Here, we discuss the role of natural hybridization in the origin of novelties using several case studies in Neotropical orchids. We highlight questions associated with the origins of morphological and ecological novelties, at different biological levels, using different spatial and temporal origins of adaptive variation contributing to novelty, and connecting the outcomes for species interactions. Does this process lead to beneficial novelties? Are they better adapted to environmental changes? Are important adaptations being lost? Natural hybridization is not the sole source of evolutionary novelty, but is perhaps the most likely mechanism of sudden, and novel, changes.

Presented by Isabel Marques ([isabel.ic@gmail.com](mailto:isabel.ic@gmail.com))

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## A pantropical population genetics study on cashew crop: uncovering genetic diversity and agrobiodiversity hotspots

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Cashew (*Anacardium occidentale* L.) is a crop currently grown in several tropical countries because of the economic importance of cashew nuts. It is assumed that in the middle of the sixteenth century the Portuguese brought the cashew tree from Brazil (center of origin) to India, and later expanded to Southeast Asia, and introduced in Africa at the same time in Mozambique. The socio-economic importance of the cashew tree in tropical regions is recognized, and in recent decades, the demand for cashew has increased at the global market. Despite its enormous economic worth, limited research has been conducted on the molecular diversity of cashew genetic resources. In this work, we present the current research performed in characterizing cashew genetic diversity under a population genetics approach, across different tropical regions, from Brazil (South America) to Guinea-Bissau and Mozambique (Africa) and East Timor, Indonesia (Asia), using CPLP countries as a case study, which also cover cashew pantropical distribution. More than 350 individual cashew trees were analyzed with 16 cashew-specific SSRs, covering a pantropical approach and a more in-depth analysis on East-Timor diversity. In terms of diversity by population, Guinea-Bissau and East-Timor populations were found to display the greatest diversity, and not the center of origin of the crop (Brazil). The population structuring revealed that genetic diversity scattering does follows a geographical trend under a continental distribution. When analyzing East-Timor populations alone, a higher allelic richness was found and population structuring analysis revealed that the genetic diversity of cashew populations in was higher than previously reported. Moreover, our study showed that cashew populations in East Timor are grouped into two dissimilar genetic groups, which may suggest multiple cashew introductions over time. Overall, our data reports the first comprehensive study on cashew intraspecific diversity using a continental approach thus highlighting the need to perform conservation programs focused on a country standpoint. This is especially important considering that the result of a limited gene flow across countries were observed, which may be due to local selection of accessions within each country. Therefore, this study provides useful information regarding genetic diversity and population structure that can be harnessed to improve cashew world production. This data is also important for creating a country-specific genetic cashew signature to increase cashew market value. Ongoing work is being developed under the Project GenoCash (PTDC/ASP-AGR/0760/2020) on associating environmental variable to genetic diversity across a pantropical distribution, to understand current genetic diversity panorama. Besides, a pangenomics study is undergoing in order to identify genome regions with regional significance that could be associated to agronomical traits performance.

Presented by Filipa Monteiro (fmonteiro@isa.ulisboa.pt)

## Plant-Microbe Interactions: Friends and Foes

Ribeiro-Barros, AR

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The evolution and diversification of plant-microbe interactions started 450 million years ago and resulted in a wide range of pathogenic and mutualistic associations. Among the later, root symbiosis with nitrogen-fixing bacteria, is among the most intriguing biological processes. This capacity is confined to two groups: legume-rhizobia and actinorhizal plants-frankia. In both cases, the interaction results in the formation of a new plant organ, the root-nodule, where bacteria are hosted and fix atmospheric nitrogen (N<sub>2</sub>). Despite several decades of research dedicated to the understanding of this exclusive type of interaction, the clue for this friendship and the ambition to transfer this capacity to crops is yet a mirage. During this presentation I will discuss some of the evolutionary aspects of root-nodule symbiosis, bringing forward the molecular mechanisms underlying nodule development and functioning, as well as the most recent phylogenomic achievements, revealing multiple losses of the plant symbiotic capacity during evolution. Acknowledgements: Fundação para a Ciência e Tecnologia through UIDB/00239/2020 (CEF) and LA/P/0092/2020 (TERRA).

Presented by Ana Ribeiro-Barros ([anaifribeiro@edu.ulisboa.pt](mailto:anaifribeiro@edu.ulisboa.pt))

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## Macaronesian Islands as “natural laboratories” to study patterns of evolution and biogeography

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Islands are of great importance for conservation of plant diversity: about 25% of the known vascular plant species are endemic to islands. The Macaronesian region (i.e., Azores, Madeira, Savage, Canary, and Cabo Verde archipelagos) is part of the Mediterranean Biodiversity Hotspot, and its flora exhibits many distinctive characteristics including a high degree of endemism (ca. 1000 species). Due to the islands' small areas and often high altitudes, they have considerable climatic variation, resulting in high ecological diversity, and so are ideal for studying evolutionary processes. The origin and evolution of the flora of the Cabo Verde archipelago is still poorly understood. Although, there are molecular phylogenies available for most of the endemic plant radiations in Macaronesia, most of them lack a good taxonomic sampling among the Cabo Verde taxa. This communication explores the relationships and evolution of some of the most widespread Cabo Verdean endemics using nuclear and plastid DNA sequences, complemented with ecological data obtained from extensive fieldwork carried out by some of the authors. We review the molecular phylogenies currently available for these plant radiations in Macaronesia and, based on new molecular data, we infer hypotheses to explain the patterns of diversity observed in the Cabo Verde endemic flora. Divergence time estimates provide a temporal framework to understand these patterns. Biogeographic patterns inferred from phylogenetic information are contrasted with those based on phytogeographic affinities. The reassessment of historical biogeography hypotheses for the Cabo Verde flora, including origin, dispersal patterns, and colonisation events are discussed, focusing on the wider geographical context of the Macaronesian Islands.

Presented by Maria Romeiras ([mmromeiras@isa.ulisboa.pt](mailto:mmromeiras@isa.ulisboa.pt))

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## Mating behaviour and fertility of a monandrous insect under thermal stress

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Insects are amongst the organisms most affected by rising temperatures, due to negative repercussions on life-history traits and, consequently, on population persistence. In many taxa males become sterile at lower temperatures than females. In these species, females could buffer the negative effect on male fertility improving their own reproductive output by rejecting sterile males or mating with more than one male. However, this rescue is conditioned by the populations' mating system and the mating behaviour of both sexes, which can also be disrupted by temperature. Unfortunately, most work has studied polyandrous species leaving a gap in our knowledge. Thus, understanding if and how monandrous species change their mating behaviour and/or mating system due to heat stress will bring important knowledge on the crucial topic of population persistence under climate warming. Here, we studied the real-time evolution of two bio-geographical distinct populations of a monandrous fruit fly (*Drosophila subobscura*) subjected to a global warming scenario. As such, we could test if selection and population history play a role in the ability to respond to temperature changes. Specifically, we aimed to assess: i) the impact of high temperatures on male mating behaviour and fertility; and ii) the impact of reduced male fertility on female mating behaviour, (in particular, propensity to remate), and fertility. We show that exposure to high temperatures leads to lower performance in males, affecting both behaviour and fertility. Importantly, these males are able to recover a functional mating behaviour through time, but not their reproductive output. We also demonstrate that monandrous females remate after exposure to heat-stressed males and that this behaviour is advantageous for the reproductive output of the females leading to a total rescue of their reproductive performance in certain conditions. Ultimately, here we bring new insights on the effects of high temperatures on mating behaviour and fertility in a monandrous species and provide a model to assess how a shift from monandry to polyandry affects species at both the individual and the population levels.

Presented by Afonso Grandela ([a.grandela@hotmail.com](mailto:a.grandela@hotmail.com))

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## The impact of environmental heterogeneity on the genomics of adaptation to cadmium

Ferreira, MC (1); Godinho, D (2); Magalhães, S (1); Sousa, VC (1); Fragata, I (1)

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Understanding adaptation in spatially heterogeneous environments is fundamental for ecology and evolutionary biology, as environmental heterogeneity plays a crucial role in governing diversity patterns across landscapes. Despite this, we still know little about this process, in particular at the genomic level. To tackle this question, we used experimental evolution of the two-spotted spider mite, *Tetranychus urticae*, evolving for 55 discrete generations in tomato plants with or without cadmium. We compared fecundity, habitat preference and SNP frequencies across the whole genome for spider mites evolving in three different regimes: (i) plants without cadmium (control); (ii) plants with high cadmium concentrations (homogeneous environment); and (iii) plants with and without cadmium (heterogeneous environment). After 33 generations of evolution, we observed an increase in fecundity in plants with cadmium for the homogeneous but not for the heterogeneous regime. Interestingly, we found significant changes in allele frequencies in SNPs along the genome, consistent with the action of positive selection in both homogeneous and heterogeneous environments. We detected signals of selection for both regimes in the *DNA2* gene, codifying an enzyme of the helicase family important for DNA replication, which was previously identified as modifying sensitivity to cadmium. However, we did not find any signs of selection in metallothioneins (a gene family involved in response to heavy metal stress). Interestingly, we detected selection for 15 chemosensory genes, belonging to the Gustatory receptor family, exclusively in the heterogeneous environment. However, we did not observe changes in cadmium preference between leaves with or without cadmium for this regime. The discrepancy observed between genomic and phenotypic responses draws attention to the need to be precise about phenotype scoring. Overall, the genomic regions under selection differed between selection regimes, suggesting that spider mites from these regimes evolved different mechanisms to detect and cope with cadmium. Our results highlight that environmental heterogeneity modulates adaptation to environmental stressors.

Presented by Marta Ferreira ([martacferreira11@gmail.com](mailto:martacferreira11@gmail.com))

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## Phenotypic and genetic variation for competitive ability and their impact on long-term interactions between spider mites

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Competition between species is a major driver of population dynamics and species coexistence. It can also affect genetic diversity, either by decreasing it if there is a strong selection for increased competitive ability, or by maintaining it. Such, within population diversity, in turn, is expected to affect the probability of coexistence. Here we aimed 1) to characterize the individual variation for intra- and interspecific competitive ability in a population of spider mites, 2) predict the impact of individual variation for competitive ability on coexistence with other species, and 3) quantify broad-sense heritability of intra- and interspecific competitive ability. For this purpose, we characterized the competitive ability of 29 *Tetranychus evansi* inbred lines originated from one outbred population and used these estimates to predict, through theoretical models, the long-term interactions with *Tetranychus urticae* (i.e., the likelihood of coexistence). Then, we estimated the broad-sense heritability for competitive ability. There was high phenotypic and genetic variation in intra- and interspecific competitive ability among the inbred lines. This individual variation translated into a plethora of possible outcomes ranging from coexistence to extinction of either competitor. This diversity suggests that traits that improve competitive ability may evolve, facilitating the population's persistence in dynamic environments and altering the probability of coexistence with heterospecifics.

Presented by Sofia Henriques (sofiasoareshenriques@gmail.com) *Eligible for best talk award*

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## Surviving the heat: How multiple mating rescues offspring sex ratio in a haplodiploid exposed to developmental heat stress

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Although survival estimates are widely used to assess the impact of temperature on organisms, reproduction is generally more sensitive to high temperatures and arguably a better predictor of the response of populations to climate change. Still, how temperature simultaneously impacts male and female reproductive success, the mating system and the operational sex ratio remains an open question. Here, we addressed how a sublethal high temperature affects the reproductive system of the haplodiploid spider mite *Tetranychus urticae*, a species where only the first mate sires the offspring of a female. Males and females maintained at 25 or 36°C during development were paired and the fertility of both sexes, their mating and remating eagerness, and the paternity of the offspring of females with different mating histories were measured. Female and male fertility declined at high temperature, resulting in lower offspring production and a more male-biased sex ratio, respectively, because of haplodiploidy. However, a shift in paternity share was observed when either heat-stressed females or females that mated with heat-stressed males remated, with more than one male contributing to the offspring. Mating behaviour accompanied this change, as mating eagerness decreased in pairs with partially sterile males, while remating eagerness increased in pairs in which at least one sex had first mated with a partially sterile partner. The observed temperature-induced changes in female remating eagerness and sperm use allowed restoring the offspring sex ratio, by increasing the proportion of fertilized offspring, but did not lead to the recovery of offspring number. These shifts in mating behaviour and mating system should alter the interactions within and between the sexes, and with it the strength of sexual selection and sexual conflict in this species. Whether such changes are sufficient to prevent population extinction remains an open question.

Presented by Sofia Costa (sofiage98@hotmail.com) *Eligible for best talk award*

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## Experimental evolution reveals a plastic response to ecological and physiological costs of suppressing plant defences

Kozak M (1), Costa SG (1), Fragata I (1), Rodrigues LR (1), Magalhães S (1)

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Herbivores that suppress plant defences have higher performance on plants with defensive compounds than those that do not have this ability. It is therefore puzzling that such characteristic is not widespread in this group. Possibly, this is due to plant defence suppression being costly, a possibility that, to our knowledge, remains untested. We hypothesized that the suppression of plant defences may entail (1) physiological costs, if the energy and resources allocated for suppression compromise herbivores' life history traits or (2) ecological costs, if the resources made available from suppression are also used by competitors. We tested these hypotheses by performing experimental evolution of *Tetranychus evansi*, a spider mite species known to suppress tomato defences, on wild-type tomato plants in (a) absence or (b) presence of competitors or (c) on tomato plants where induced defences are impaired (def-1 plants), in the absence of competitors. After 16, 18 and 26 generations of experimental evolution, we measured fecundity and plant suppression of mites evolving in these selection regimes, when coming directly from the environments on which they were selected. We compared this response to that of mites reared in a common environment (on wild-type tomato plants), this way equalizing maternal effects, after 35 and 37 generations of experimental evolution. When coming directly from the selection lines, *T. evansi* populations that evolved on def-1 plants or on wild-type plants in the presence of competitors suppressed plant defences to a lesser extent than populations evolving on plants with intact defences and no competitors. This suggests that there are both physiological and ecological costs of defence suppression. However, these responses were not observed when the evolved populations were exposed to a common environment, which suggest that the response of mites to the environment (both competitors and plants) is plastic and relies on maternal effects. Therefore, whether herbivores pay a cost of suppressing plant defences may hinge upon how often they switch environments.

Presented by Mariya Kozak (makozak@ciencias.ulisboa.pt) *Eligible for best talk award*

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## How does ecological stability affect evolution?

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Predator-prey interactions are prevalent in many systems and are a well-known example of how eco-evolutionary dynamics may affect species interactions. However, we still know very little about the impact of the stability of species interactions on subsequent evolutionary trajectories. To tackle this problem, we assess how the evolution of different predator and prey traits might affect the stability of coexistence, using a Rosenzweig-MacArthur predator-prey model with two types of predator avoidance mechanisms (defense and dispersal). In this talk, I focus on the effects of predator evolution, and determine the parameter space that enables stable coexistence. We find that a simple predator fitness landscape with a fitness optimum can be traced from the ecological dynamics, for predator traits such as attack rate and handling time. Along this fitness landscape, we are able to identify the parameter space that allows for stable coexistence and whether the optimum lies within it. In addition, using perturbation analysis, we determine whether the stability of the system is resilient to random deviations from the optimum and assess a potential trade-off between evolving towards a fitness optimum and being less resilient to evolutionary perturbations (i.e. higher risk of extinction). These results highlight how factors such as ecological stability and resilience to evolutionary perturbations might affect fitness landscapes, and demonstrate the importance of ecological context in determining evolutionary trajectories.

Presented by Jessica King ([jaking@fc.ul.pt](mailto:jaking@fc.ul.pt))

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## The population bottleneck of the Iberian wolf impacted genetic diversity but not admixture with domestic dogs: a temporal genomic approach

Lobo, D (1,2,3); López-Bao, JV (4); Godinho, R (1,2,3,5)

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After decades of intense persecution, the Iberian wolf subspecies faced a severe bottleneck in the 1970s that considerably reduced its range and population size, nearly leading to its extinction in central and southern Iberian Peninsula. Such population decline could have impacted the genetic diversity of Iberian wolves through different processes, namely genetic drift and dynamics of hybridization with domestic dogs. By contrasting the genomes of 68 contemporary with 54 historical samples spanning the periods before and immediately after the 1970s bottleneck, we found evidence of its impact on genetic diversity and dynamics of wolf-dog hybridization. Our genome-wide assessment revealed that wolves and dogs form two well-differentiated genetic groups in Iberia, and that hybridization rates did not increase during the bottleneck. However, an increased number of hybrid individuals was found over time during the population re-expansion, particularly at the edge of the wolf range. We estimated a low percentage of dog ancestry (~1.4%) in historical samples, suggesting that dog introgression was not a key driver for wolf extinction in central and southern Iberia. Our findings also unveil a significant decline in genetic diversity in contemporary samples, with the highest proportion of homozygous segments in the genome being recently inherited. Overall, our study provides unprecedented insight into the impact of a sharp decline on the Iberian wolf genome and refines our understanding of the ecological and evolutionary drivers of wolf-dog hybridization in the wild.

Presented by Diana Lobo ([diana.lobo@cibio.up.pt](mailto:diana.lobo@cibio.up.pt))

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## Multiple mitochondrial introgression events from an ancient lineage of Rüppell's fox (*Vulpes rueppelli*) into red fox (*Vulpes vulpes*)

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The mitochondrial introgression has been reported between red foxes (*Vulpes vulpes*) and Rüppell's foxes (*Vulpes rueppellii*), two closely related sister species that differ fundamentally in ecology, morphology and physiology. Despite mainly inhabiting different environments, both species overlap their ranges in semi-arid regions in the North Africa and Middle East. The evolutionary scenario of old divergence and recent introgression between these two species has been proposed but the directionality and time of this event remains unclear. To further investigate this scenario, we analysed 86 mitogenomes of both red and Rüppell's foxes distributed across their ranges. We confirmed the paraphyly of red and Rüppell's foxes, with three clades of red foxes sampled in Tunisia, Iran and, Iran and Turkey grouping with Rüppell's foxes. Time estimates suggest that Rüppell's fox and introgressed lineages have diverged around 0.309 mya (95% HPD=0.059–0.811 mya), following the divergence of both species around 0.696 mya (95% HPD=0.152-1.918 mya), but prior the recent intraspecific divergence of Rüppell's fox around 0.077 mya (95% HPD=0.002-0.314 mya). While supporting the old divergence of both species, our findings suggests that introgression likely occurred from an ancient lineage of Rüppell's fox into red fox. Multiple hybridization events would have occurred for this pattern prevail across several localities, mainly at the southern fringe of the distribution of the red fox. Further, introgression appears to have occurred unidirectionally, that is, from the desert-adapted species – Rüppell's fox – into the temperate species – red fox, which may be explained by differential ecological conditions favouring the contact, asymmetrical reproductive behaviour, and/or selective advantage promoting introgression.

Presented by Rita Rocha (ritagomesrocha@gmail.com)

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## The value of captive wildlife in the conservation of species: Exploring wild origins and genetic diversity in the iconic sable antelope

Meneses-Ribeiro, M (1, 2, 3); Jansen van Vuuren, B (4), Koepfli, KP (5, 6); Godinho, R (1, 2, 3, 7)

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As defined by the IUCN, a species' conservation status considers population numbers both in the wild and in ex-situ captive populations. Although genetic diversity is not formally included in these classifications, it may be pivotal to optimal conservation management. A case in point is the sable antelope (*Hippotragus niger*), whose population numbers are dwindling in the wild, but occurs in high numbers in captivity, especially on privately owned farms and ranches. Sable is a large African antelope that comprises five different phylogroups. The species is classified as "least concern", despite the current paucity of evaluation of genetic diversity. This study seeks to understand the importance of monitoring and preserving genetic diversity in captive animals that could act as a reservoir for the restoration of wild populations. For that, we evaluated genetic diversity, inbreeding and admixture in wild and captive populations using microsatellite markers in 7,900 captive sables from 358 farms, representing one of the largest datasets for a wildlife species analyzed to date, and compared results with 320 wild specimens. Results revealed that only two phylogroups were present in captive populations, with 80% of the animals being admixed. Notably, pure phylogroup animals are restricted to a few farms making the future conservation of sable dependent of a scarce number of private owners. Furthermore, farm populations showed decreased genetic diversity and higher relatedness and inbreeding relative to wild populations. Despite the high number of captive animals, results suggest that a small fraction can truly represent wild populations, which negatively impacts future conservation planning. Our study demonstrates the usefulness of comprehensive genetic analyses of captive populations that can serve as stocks for the conservation of wild species.

Presented by Mariana Ribeiro (mariana.ribeiro@cibio.up.pt) *Eligible for best talk award*

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## Table for two: diet composition differences of allopatric and sympatric populations of island geckos

Pinho, CJ (1,2,3); Santos, B (1,3); Mata, VA (1,3); Lopes, RJ (1,3,4); Romeiras, MM (5); Lopes, EP (1,3,6); Alves, PC (1,2,3); Emerson, BC (7); Vasconcelos, R (1,3)

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Oceanic islands are exciting models for studying how evolutionary processes and environmental variables can jointly contribute to speciation and community assembly over time. In this context, the Cabo Verde archipelago serves as a simplified natural laboratory, harbouring a group of endemic reptile species that descended from a single common ancestor, with sympatric species exhibiting wide morphological variation, particularly for size, and competing for limited food resources. This framework allows for the exploration of how diet composition of closely related and geographically overlapping species is influenced by competition. This is exemplified by the two endemic wall geckos, *Tarentola gigas* and *T. raziana*, which occur in sympatry on Raso Islet, with the latter also occurring in allopatry on Santa Luzia Island. DNA metabarcoding of faecal pellets was used to compare the diets of both sympatric and allopatric populations, revealing significant differences in diet composition between both populations of *T. raziana*, with significantly lower prey richness and niche breadth in the Santa Luzia population. Differences observed in sympatric species were due to a higher incidence of vertebrates and plants in the diet of *T. gigas*, while differences between the allopatric populations of *T. raziana* were due to a higher incidence of plants and a lower incidence of invertebrates in the population of Santa Luzia, compared to Raso. These results suggest that competition has played an important role in structuring the stable coexistence of these species in sympatry, promoting extreme morphological discrepancies that allowed resource partitioning. In addition, this study reveals unexpected dietary specialization within the allopatric population of *T. raziana* on Santa Luzia, suggesting a niche shift in response to the absence of a direct competitor. Furthermore, this work highlights the importance of understanding the mechanisms of resource partitioning for conservation efforts and management, especially for fragile island ecosystems.

Presented by Catarina de Jesus Pinho (cjpinho@cibio.up.pt) *Eligible for best talk award*

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## Signatures of adaptation at the mitochondrial level in endemic freshwater fish species from contrasting environments

Bernardo, C (1,3); Mendes, S (1,3); Moreno, J(1,2,3); Sousa, V (1,3)

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Temperature is an important selective pressure, especially for ectotherms that depend on external environment to maintain homeostasis, making them vulnerable to small environmental changes. Temperature variations can trigger responses at the ATP metabolism level, regulated by the mitochondria. Detecting mitochondrial genes involved in adaptation can elucidate key environmental selective pressures and their effects on metabolism. The *Squalius* genus is obligatory freshwater fish inhabiting different environments. In the Iberian Peninsula, there are six diploid species with distinct thermal environments, characterized by increasing temperatures and drought propensity from north to south. Moreover, *Squalius alburnoides*, an allopolyploid hybrid complex, is found in sympatry with most of the other *Squalius* species. Here, to reconstruct the phylogenetic relationships between species and detect genes under selection potentially due to temperature and metabolism, we compared the whole mitochondrial genome sequence of diploid *Squalius* species from different environments, as well as with sympatric *S. alburnoides*. We performed a de novo assembly and annotation of the mitochondrial genome for each of the Iberian chub species. The whole mitochondrial genome phylogeny presents three major clades, such as previous results. *S. alburnoides* mitochondrial sequences do not form a monophyletic clade. Instead, they generally cluster with the sympatric species, supporting local introgression of mitochondria between local diploid species and sympatric *S. alburnoides* populations. Then, to detect molecular signatures of selection we applied dN/dS-based tests which showed the existence of signals of selection, both positive and negative, associated with protein thermostability. The selection test results found a common site under positive selection for *S. carolitertii* and *S. alburnoides* from Tua River, suggesting that *S. alburnoides* and its sympatric species could be under the same selective pressures.

Presented by Catarina Bernardo (fc52632@alunos.fc.ul.pt) *Eligible for best talk award*

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## The molecular bases of red-to-yellow color variation in parrots

Arbore, R

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Parrots produce stunning plumage colors by the endogenous synthesis of a unique class of pigments known as psittacofulvins. A polyketide synthase has been shown to be required for psittacofulvin biosynthesis, but the molecular mechanisms explaining variation in psittacofulvin coloration remain to be elucidated. We found that red and yellow colors in parrots result from the differential deposition of aldehyde and carboxylic-acid psittacofulvin forms during feather development. Then, we integrated genetic mapping with gene expression and functional experiments to identify an aldehyde dehydrogenase as the key regulator of a red/yellow polymorphism in wild parrot populations. Finally, through fine mapping and single-cell genomic techniques (scRNA-seq and snATAC-seq) we traced this polymorphism to a single point mutation in a conserved regulatory element exclusive to differentiating keratinocytes in developing feathers. The simplicity of the proposed genetic and enzymatic mechanisms offers an explanation for the exceptional evolutionary lability of psittacofulvin-based colors throughout parrot evolution. Our study illuminates the molecular mechanisms governing the colorful plumage of parrots, offering insights into the evolutionary origins of key innovations via the recruitment of genes with essential cellular functions.

Presented by Roberto Arbore ([roberto.arbore@gmail.com](mailto:roberto.arbore@gmail.com))

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## Abandoned gold mine reveals new mysterious nuggets: Redescription of three fossil whales from the Miocene of Portugal

Figueiredo, R (1); Bosselaers, M (2,3); Póvoas, L (4); Castanhinha, R (1,5)

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Understanding the phylogeny of the different families of fossil cetaceans give us insights into the evolution and adaptations that emerged over millions of years in the group of the largest animals that ever existed, from the most primitive semiaquatic protocetids to the whales that we see today crossing the oceans. Since its first description, the Cetotheriidae family has been controversial, and in the past two decades there have been various studies attempting to solve the phylogeny of this group. In 1831, Alexandre Vandelli collected three cetotheriid skulls, found during a gold exploration at Adiça beach (Portugal). These specimens constituted the first Portuguese vertebrate fossils described in the literature. In 1914, during a survey of the Museu Nacional de História Natural collections, Jacinto Pedro Gomes refers that there is another skull associated among the “Vandelli skulls”. Later, in 1941, Remington Kellogg adds more mystery stating that one of the skulls referred by Vandelli is no longer present in the Museu Nacional de História Natural collections. Until today, how and exactly when these skulls appeared and disappeared remains unexplained. All previous attempts to describe these specimens were not based on direct observations and no comprehensive phylogenetic analysis has included the skulls still available. The present study provides a detailed anatomic description, an up-to-date phylogenetic analysis and a palaeoecological reconstruction of these specimens, clarifying their relationships within the Cetotheriidae family and fostering the importance of these historical specimens to the modern comprehension of fossil whale evolution. In addition, the results of this study support that *Cephalotropis nectus* is a valid species with an emended diagnosis, and describe a new genus for the other “Vandelli skulls”, forming two new fossil species (new combinations).

Presented by Rodrigo Figueiredo (rodfigueiredo10@hotmail.com)

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## Phenotypic plasticity in response to climate change in Iberian chubs: insights from proteomics and phosphoproteomics

Moreno, JM (1,2); Grossmann J (3,4); Kunz, L (3); Sousa, VC (1); Santos, R (2)

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Climate change is drastically affecting freshwater ecosystems across the globe. Fish can be greatly affected by changes in water temperature, but also in other water conditions such as pH. Here, we study the effects of water temperature and acidification at the proteome level in two Iberian freshwater chubs from the *Squalius* genus (*S. carolitertii* and *S. torgalensis*) from different lineages and environmental backgrounds. After exposing the fish to different scenarios of climate change (warming, acidification, and a combined scenario) we analyzed the differential proteome and phosphoproteome from gills and muscle. Our results suggest a higher number of differentially expressed proteins in fish inhabiting warmer environments (*S. torgalensis*) compared to fish from colder environments (*S. carolitertii*). These proteins are mainly associated with histone regulation and collagen-related response in the gills and protein folding and stress-related pathways in the muscle (traditionally associated with response to temperature). Furthermore, our results revealed differences at the phosphoproteome level with a higher level of differential phosphorylation compared to the total proteome in both *S. carolitertii* and *S. torgalensis*. While in *S. carolitertii* these differences appeared to be in muscle-related proteins, in *S. torgalensis* the differential phosphorylation was found to be in proteins associated with energy metabolism and regulation of gene expression, in particular mRNA processing. The more pronounced levels of differential phosphorylation compared with the differential protein expression may indicate that regulation through phosphorylation may have a lower cost for the organism compared to modulating the whole expression, and thus be a more cost-effective mechanism to cope with environmental changes. Altogether, these results suggest that, in these species, the response to temperature and water acidification relies on both the modulation of protein expression and its post-translational regulation, mainly in important biological processes such as metabolism, folding, and gene expression regulation. Moreover, we present here the utility of combining proteomics and phosphoproteomics as a tool to study the effects of climate change at the molecular level.

Presented by João Moreno (jmmoreno@fc.ul.pt) *Eligible for best talk award*

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## Contrasting outcomes of hybridization in Iberian chubs revealed through whole genome data

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Hybridization is increasingly acknowledged as a force generating diversity through the exchange of alleles between lineages. Combination of alleles previously in separate genomic backgrounds and the exchange of adaptive variants have been shown to promote diversification and adaptation in several organisms. However, hybridization can also generate maladaptive combinations or lead to the disappearance of species through merging. The Iberian chubs (genus *Squalius*) are a group of freshwater fish with a mostly allopatric distribution across distinct environments and river types. Throughout their evolutionary history, the river basins they inhabit suffered quite dramatic changes, fueling multiple periods of isolation and secondary contact. They are currently distributed along an environmental cline of increasing temperature and propensity for drought from north to south, subjecting different lineages to distinct environments. We generated whole genome data from 125 individuals covering the distribution of six species and used this to investigate the prevalence and consequences of past and ongoing hybridization in the group, focusing particularly on *S. carolitertii* and *S. pyrenaicus*. Our testing uncovered three contrasting cases of hybridization. First, we detect ancient admixture between two species at the origin of a third lineage. Second, we uncover recent, possibly ongoing, hybridization between two lineages of *S. pyrenaicus*. Finally, we detect a third case of hybridization where two very distinct mitochondrial lineages persist despite seemingly uniformization at the nuclear level. These results show the Iberian chubs are an excellent system to study the different possible outcomes of interspecific hybridization, and its role in both generating and reducing biodiversity.

Presented by Sofia Mendes (slmendes@fc.ul.pt) *Eligible for best talk award*

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## Density-dependent dispersal promotes female-biased sex allocation and reduces sex-ratio conflict in viscous populations

Chedhawatt Chokechaipaisarn (1); Andy Gardner (1)

CBD - centre for biological diversity, School of Biology, University of St Andrews, UK

One major prediction of sex allocation theory is that the optimal sex ratio is completely independent to the rate of dispersal. Although reduced dispersal had previously been expected to promote female bias, by increasing relatedness within the group, it also leads to an intensification of local competition for resources, which tends to inhibit female bias—and these two effects exactly cancel each other out. This remarkable invariance result has stimulated a huge amount of attention into social evolution in viscous populations. However, it is crucially based upon the assumption that individuals do not modify their dispersal behaviour according to local population density. Here, we investigate how density-dependent dispersal shapes patterns of sex allocation in a viscous-population setting. We find that if individuals are able to adjust their dispersal behaviour according to local population density, then they are favoured to do so, and this promotes the evolution of female-biased sex allocation by disentangling the relatedness and kin-competition consequences of limited dispersal. Moreover, we apply a battleground approach to investigate how density-dependent dispersal impacts upon the evolution of maternally versus paternally controlled sex allocation. Although under haploid and diploid modes of genetic inheritance there is no conflict between parents over the sex ratio, we find that under haplodiploidy the degree of conflict between mothers—who favour a less female-biased sex ratio—and fathers—who favour a more female-biased sex ratio—is lower when dispersal is density-dependent.

Presented by Chedhawatt Chokechaipaisarn (cc385@st-andrews.ac.uk) *Eligible for best talk award*

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## Interactions between mechanisms of reproductive isolation

Blanckaert, A(1); Sousa, VC (1)

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Speciation is the mechanism responsible for the incredible diversity of species that we observe today and corresponds to the build-up of reproductive isolation between different populations. Reproductive isolation itself can be generated by different mechanisms, for example ecological differentiation, mate choice or sterility of hybrids. Understanding how each of these mechanisms acts individually has been the focus of many evolutionary studies, yet how their interactions affect speciation remains largely unknown. We model two populations exchanging gene flow to quantify the strength of reproductive isolation that will evolve under different conditions, considering what is caused by each mechanism (local adaptation, mate choice, intrinsic hybrid load) and their interaction. More precisely, we use Fisher Geometric Model to model local adaptation, phenotype matching for mate choice and Bateson Dobzhansky Muller Incompatibilities for intrinsic hybrid load. All three barriers are affected by the same set of loci, creating conditions for interactions between barriers both at the genetic and population levels. Initial results show that mate choice may evolve in response to the presence of postzygotic isolation, or directly to the ecological gradient experienced by the two populations, depending on the strength of the latter. In addition, we show that the life cycle and more precisely the life stage at which migration happens can strongly impact the evolution of mate choice. Finally, we also observe that mate choice alone may have a counterintuitive effect, favoring the introgression of neutral markers, from one population to the other.

Presented by Alexandre Blanckaert ([blanckaert.a@gmail.com](mailto:blanckaert.a@gmail.com))

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## Human migrations and gene flow with local dogs shaped the genomic ancestry of livestock guarding dogs across Eurasia

Diogo Lima (1,2,3), Dayna L. Dreger (4), Ignacio Doadrio (5), Heidi G. Parker (4), Laurent Frantz (6,7), Greger Larson (8), Elaine A. Ostrander (4) & Raquel Godinho (1,2,3)

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Livestock guarding dogs (LGDs) are large dog breeds primarily selected to protect livestock from predation. Despite their importance to human welfare, our understanding of LGDs genetic diversity, breed differentiation and relationships with other dog populations is limited. Moreover, whether LGD breeds have a common or multiple origins remains unanswered. To address these questions, we investigated the genomic structure, ancestry, and phylogenomic relationships amongst LGDs throughout Eurasia. We used genome-wide SNP data from a diverse set of over 300 LGDs that represent 36 breeds, and 175 contemporary Eurasian free-ranging dogs, together with 22 ancient dogs. Our findings reveal a prevalence of mixed ancestry and extensive gene flow among modern LGD breeds within close geographic regions, which we associate with human migrations. Additionally, we observe signals of gene flow between LGDs and local free-ranging dogs, not favouring effective reproductive isolation as a core mechanism used to maintain the specialized skill set of LGDs. Finally, we uncover a polyphyletic pattern through phylogenomic analyses, revealing the presence of two contemporary lineages within LGDs with a clear geographic structure, each rooted to the expected local origins of the breeds they comprise. This dual pattern of ancestry has been significantly shaped by distinct human migrations and dogs' ancestry replacements across time. Overall, our work represents the first investigation into the intricate evolutionary history of LGDs, providing significant insight and to how human and livestock migrations shaped the genetic background of these working dogs.

Presented by Diogo Lima (diogofclima5@cibio.up.pt) *Eligible for best talk award*

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# ABSTRACTS OF POSTER PRESENTATIONS

## Poster 1

### First record of the *Hemidactylus mabouia* gecko for the island of Sal, Cabo Verde

Almeida, L (1,2,3); Vasconcelos, R (1,2)

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It was found that invasive species disturb the relationships between endemic species and the ecosystem, through competition for resources, predation, disease transmission, among others, and reptiles are among the most affected groups. Island endemics generally present increased risks of extinction by invaders. On the Cabo Verde Islands, reptile species have already gone extinct in part due to invaders. The *Hemidactylus mabouia* is a gekkonid introduced in Cabo Verde and must be closely monitored to ensure that it does not expand its distribution or displace native species. This was already the case of the *Hemidactylus angulatus*, also introduced in Cabo Verde, with the native *Hemidactylus boavistensis*. With this aim, reptiles were searched under the rocks during the day and on the ground when active at night in the central part of Sal Island, Cabo Verde. Invasive reptiles were photographed, GPS recorded, and a tissue sample collected. DNA was extracted from the tail tissue using the saline method and amplified using universal 12S primers. Amplified products were Sanger-sequenced. Pictures of the diagnostic morphological characters and the genetic sequencing confirm animals belong to the *H. mabouia* species. Before this work, the distribution of *H. mabouia* in Cabo Verde was confirmed only in three islands: Santo Antão, Brava and São Vicente. However, this work confirmed, for the first time, its occurrence on Sal, which means that the species continues to expand its distribution in the country. It also showed that it has the same haplotype as the other islands, that had been proved to correspond to introduction. More studies are needed on the effects of the presence of this species on the archipelago and quick measures to prevent its expansion among islands, in order to guarantee the protection of the endemic species.

Presented by Lara Almeida (laralmeida2195@gmail.com) *Eligible for best poster award*

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## Poster 2

### Thermal plasticity in butterfly pigmentation depends on genetic and environmental context

Atencio, G(1); van Bergen, E(1); Saastamoinen, M(2); Beldade, P(1)

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Phenotypic variation in natural populations results from complex interactions between organisms and their changing environments, with the environment shaping both phenotypic frequencies (during adaptation) and organismal phenotypes (through phenotypic plasticity). Developmental plasticity, in particular, refers to the phenomenon whereby an organism's phenotype depends on the environmental conditions during development. It can match phenotype to ecological conditions and help organisms cope with environmental heterogeneity, including differences between alternating seasons. Experimental studies of developmental plasticity often focus on the impact of individual environmental cues on one or a few distinct genotypes. However, natural environments are much more complex, varying for multiple environmental factors whose combined effects on phenotype might not be additive and might vary between genotypes. The African tropical satyrid butterfly *Bicyclus anynana* (Butler, 1879) is a species that exhibits seasonal polyphenism. These butterflies live in habitats with alternating dry and wet seasons that differ in humidity and temperature, as well as in quality and availability of food plants. The seasonal morphs differ in wing pigmentation associated with distinct seasonal strategies to escape predators. During laboratory rearing, dry- and wet-season phenotypes can be induced by lower and higher developmental temperatures, respectively. While we know about the phenotypic effects of temperature and their physiological underpinnings, our understanding of how thermal plasticity in pigmentation varies in response to food availability and across genotypes remains limited. To address this, we investigated the combined effects of temperature (T; two levels: 20°C and 27°C) and food availability (N; two levels: control and limited) during development. We examined their impact on eyespot size in adult males and females from multiple genotypes (G; 28 families). We found significant effects of food quantity (N effects), and significant non-additive interactions between food and temperature (TxN effects). Food limitation resulted in relatively smaller eyespots and tempered the effects of temperature. Additionally, we found differences among families for thermal plasticity (significant GxT effects), but not for nutritional plasticity (non-significant GxN effects) nor for the combined effects of temperature and food limitation (non-significant GxTxN effects). Our results uncovered previously unknown nutritional plasticity for *B. anynana* eyespot size, and revealed the context dependence of thermal plasticity in this trait, with the slope of thermal reaction norms varying across genotypes and across nutritional environments.

Presented by Guilherme Atencio ([gwatencio@ciencias.ulisboa.pt](mailto:gwatencio@ciencias.ulisboa.pt))

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### Poster 3

## Evolution of Reproductive Isolation in two spider-mite species in the presence of Local Adaptation

Barreto, S (1); Grandela, A (1), Cruz, MA (1); Sousa, VC (1); Magalhães, S (1); Zélé, F (2); Fragata, I (1); Blanckaert, A (1);

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Understanding how speciation occurs requires the study of reproductive isolation and of its evolution. Different mechanisms (e.g., local adaptation, mate choice, hybrid load) may contribute to the genetic barriers that exist between different species. Therefore, it is important to characterize the role of the different reproductive barriers, how they arise and strengthen, and the potential interactions between the different mechanisms. This latter point has received little attention. To address this point, we will follow the evolution of different reproductive barriers in the presence of gene flow and/or ecological differentiation between two nascent species of spider mites (*Tetranychus cinnabarinus* and *Tetranychus urticae*), by performing 10 generations of experimental evolution. This will be done by creating selection regimes with: 1) no migration between populations, 2) migration between populations inhabiting and adapted to the same host species (bean plant), and 3) migration between populations inhabiting and adapted to different hosts (bean plant and tomato plant). The strength of the different reproductive barriers will be characterized before and after experimental evolution by measuring local adaptation (i.e., female fitness in their environment), hybrid load, and male mate choice. Preliminary results reveal the existence of strong postzygotic isolation and incomplete mate choice between the two species. This setup will allow us to test how mate choice evolves between populations who are adapted to different environments and whether ecological differentiation promotes stricter conspecific preference. In addition, we will be able to assess whether migration triggers the evolution of reinforcement and whether evolution of mate choice has an associated fitness cost. With this project we will gain insights into the impact of the environment on the emergence of additional genetic barriers, mainly behavioral prezygotic barriers, and eventually of new species and uncover more of the inner workings of the mechanisms that contribute to speciation.

Presented by Salomé Barreto ([salomeb.barreto@gmail.com](mailto:salomeb.barreto@gmail.com)) *Eligible for best poster award*

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## Poster 4

### How do biochemical and environmental factors shape the fitness landscape of synonymous mutations on Hsp90?

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Synonymous mutations (i.e. codon changes that do not affect the amino acid coded) have historically been considered neutral. However, several studies have shown that this is not always the case. Several mechanisms can be underlying these changes, e.g. codon bias, changes in mRNA structure, stability, or co-translational protein folding. In addition, these mechanisms can be influenced by several internal or external factors, such as the regulation of protein expression by different promoters, the functional role of the protein domains, or environmental stresses. This project aims to understand how the interaction between external and internal factors impacts the fitness effects of synonymous mutations. Because of its role in several stress responses, the chaperone Hsp90 of *Saccharomyces cerevisiae* is a good candidate to investigate these intricate interactions and quantify their effect on fitness. We will use data previously obtained through Deep Mutational Scanning to estimate the fitness effects of all possible codon changes for each position of Hsp90 across several environments – including nutrient deficiency, thermal, osmotic, and oxidative stresses - and two expression levels (high and low). Firstly, we will assess the distribution of the fitness effects of synonymous mutations across different protein domains to select candidate mutations with stronger fitness effects. Next, we will compare the strength of candidate mutations across environments to quantify the role of external factors in the fitness effect of synonymous mutations. In a third step, we will test the impact of the different expression levels on the strength of synonymous mutations and how they interact with external factors (i.e. the different environments). Finally, we will analyze other deep mutational scanning data sets to assess the impact of synonymous mutations on different proteins and test if the patterns found in Hsp90 are similar to other proteins. With this project, we aim to study how various factors and mechanisms impact the fitness landscape of synonymous mutations, which will help better understand the role of synonymous mutation in adaptation.

Presented by Diogo Berjano (dnaberjano@gmail.com) *Eligible for best poster award*

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## Poster 5

### Master of Computational Biology & Bioinformatics

**Braga, A (1); Tobias, PA (2); Várzea, V (1,3); Morais-Cecílio, L (1); Park, RF (4); Ramos, MJN (5); Batista, D (1,3)**

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Screening for evolutionary changes related with virulence in the coffee leaf rust pathogen (*Hemileia vastatrix*)

*Hemileia vastatrix* (Hv) is a biotrophic fungus that causes the main Arabica coffee disease (coffee leaf rust - CLR), following a gene-for-gene host-pathogen relationship. Through this dynamic interaction, Hv have spread to all continents, easily adapting to new coffee resistances deployed in the field by breeding programs and leading to recurrent epidemics. This high adaptive capacity of Hv is quite unusual considering the apparent lack of sexual reproduction. Since coffee production is an industry that generates 70 billion US dollars per year, being crucial for economy of more than 50 countries, CLR outbreaks can cause losses of over \$1 billion annually worldwide (Silva et al., 2022). Understanding the evolutionary mechanisms of host adaptation and virulence differentiation is crucial for disease management. In this work, we are using 10 Hv isolates collected worldwide, with contrasting virulence profiles, to understand the appearance of genomic variations like SNPs and structural polymorphisms that may identify evolutionary patterns related with increased virulence emergence. We also aim to understand how a supposedly asexual pathogen can generate high levels of genetic variability and so quickly. To address these questions, we mapped Illumina reads of each isolate onto a chromosome-level Hv reference genome, recently made available (Tobias et al., 2022) and performed a variant calling to detect genomic profiles putatively linked with the different pathotypes. Since Hv is dikaryotic, we expect to find differences between the two haploid nuclei that may be involved in virulence differentiation, particularly related with effector gene content. This study is of extreme importance for the understanding of Hv evolution, focusing on how this fungus generates such a high level of genetic variability and evolves virulence so rapidly.

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Presented by Ângelo Braga (bragangel@gmail.com) *Eligible for best poster award*

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## Poster 6

### Evolutionary patterns in *Aeonium* (Crassulaceae): relationships between genome size and extinction risk

Brilhante, MA (1,2); Roxo, G (1,3); Catarino, S (1,4); dos Santos, P (2); Menezes Sequeira, P (5); Reyes-Betancort, JA (6); Caujapé-Castells, J (7); Talhinhos, P (1) & Romeiras, MM (1,2)

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The Macaronesia Islands are among the top hotspots for biodiversity protection in the world due to their rich unique flora. The radiations of endemic species that fill the most diverse ecological niches in a limited distribution area are the outcome of the diversification of insular lineages. The Canary Islands' most speciose radiation, comprising about 47 taxa, is the genus *Aeonium* (Crassulaceae). As such, it provides an ideal model for studying the dynamics of the eco-evolutionary processes that underlie the diversity of the insular flora. Through this study, we aimed to estimate the genome size (GS) variation in *Aeonium* in Macaronesia (i.e., Madeira, Canary Islands, and Cabo Verde) and determine its correlations with information on distribution, morphology, and preferred habitats. By means of an extensive fieldwork, the GS (2C-value) of 27 *Aeonium* species across Macaronesia was assessed. Furthermore, the evaluation of species' conservation status was conducted, and the findings showed that 67% of the *Aeonium* are threatened (10% Endangered and 57% Vulnerable). Also, for the first time, the GS of a comprehensive sample of *Aeonium* of Macaronesian archipelagos was estimated. Significant differences were observed, with GS ranging from 0.984 pg (*A. dodrantale*) to 2.768 pg (*A. gorgoneum*). Our results revealed a positive relationship between GS and conservation status, with larger genomes being associated with threatened species. However, there were only slight variations between genome size and morphological traits, and habitat preferences. Finally, combining genome size and the *Aeonium* IUCN Red List assessments will contribute with new data, both to implement new conservation priorities for species and archipelagos and to improve our understanding of *Aeonium* diversification in Macaronesia islands.

Presented by Miguel Ângelo Brilhante (mbrilhante@isa.ulisboa.pt) *Eligible for best poster award*

## Poster 7

### Gene pool as a conservation and evolutive tool: a case study of Mozambique's *Vigna*

Brilhante, MA (1,2); Catarino, S (1,3); Darbyshire, I (4); Bandeira, S (5); Moldão, M (1); Duarte, MC (2) and Romeiras, MM (1,2)

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Mozambique is home to great number of native legume species, including several Crop Wild Relatives (CWRs), which could be important for the long-term sustainability of regional agricultural systems. Although currently understudied, the *Vigna* genus stands out among these legumes as a promising donor of advantageous traits for crop enhancement. Through this study, we aimed to provide a current overview of *Vigna* CWRs diversity in Mozambique, unveiling their potential as providers of useful traits for crop enhancement, as elements for the improvement of agricultural systems, and to define a conservation strategy for priority target taxa and areas. Thus, a checklist of *Vigna* taxa (including details on their native status in Mozambique, habit, native distribution, conservation status, habitat, gene pool, and main uses) occurring in Mozambique was prepared using thorough literature research, herbarium collections, and online databases. The prioritization of each *Vigna* taxa in terms of conservation was performed based on five criteria (i.e., taxonomic group/gene pool, ethnobotanical value, global and regional distribution, and ex situ conservation). Our results stated the occurrence of 20 *Vigna* native taxa across Mozambique, with Manica, Maputo, and Tete provinces being the diversity hotspots. Thirteen taxa were categorized as Least Concern (LC) according to IUCN guidelines, while the remaining seven were listed as Not Evaluated (NE). With less than 10 accessions, only eight taxa (35%) had accessions in germplasm banks. We prioritized for further conservation action in three groups of priority: high (25%), medium (65%), and low (10%). According to *the in situ* gap analysis, most of the *Vigna* taxa were recorded outside of Mozambique's protected areas network, being unprotected. By providing new resources and knowledge for sustainable use in crop improvement as well as strategies for further conservation efforts, this study will advance our understanding of *Vigna* CWR taxa in Mozambique.

Presented by Miguel Ângelo Brilhante (mbrilhante@isa.ulisboa.pt) *Eligible for best poster award*

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## Poster 8

### Exploring the diversification of African Fabaceae trees in Miombo and Mopane woodlands

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Tropical dry forests, such as the Miombo and Mopane woodlands, play a crucial role in supporting millions of livelihoods through the provision of essential resources like timber, food, forage, and medicines. These ecosystems, dominated by Fabaceae species, are increasingly threatened by overexploitation, pollution, and intense fires, requiring more conservation initiatives. Despite their importance, the phylogenetic diversity of these ecoregions remains understudied, emphasizing the need to understand and characterize the evolutionary history of tree legumes for effective conservation and sustainable management. In this study, a comprehensive database of native tree legume species from the Mopane and Miombo woodlands was compiled through extensive research in scientific publications and online databases. Scientific names were updated, and additional information on distribution, species' habit, conservation status, and molecular data were gathered from diverse sources. For molecular analysis, three markers, including chloroplast genes *rbcL* and *matK*, and the internal transcribed spacer (ITS) region, were selected. Phylogeny reconstructions were conducted using two methodologies, namely maximum likelihood (ML) and Bayesian inference (BI). In both cases, species from the Polygalaceae family, a sister group of Fabaceae, were selected as outgroups. The study identified 78 tree species, belonging to the Fabaceae family, with 74 occurring in the Miombo woodlands and 23 in the Mopane woodlands, including 19 species common to both habitats. Zambia exhibited the highest species diversity (91% of the species), while the Democratic Republic of Congo had the lowest (65%). In the Miombo woodlands, five subfamilies were observed, with Caesalpinioideae being the most prevalent (27 species), while the Mopane woodlands held four subfamilies. The conservation status of most studied species has recently been assessed, with approximately 83% listed on the International Union for Conservation of Nature (IUCN) Red List. Among the evaluated species, 63 are classified as least concern (LC), one as near threatened (NT), and one as vulnerable (VU). Due to the unavailability of DNA sequences for all selected taxa, the study focused on 67 tree species for phylogenetic analysis. Phylogenetic trees obtained using the *matK* matrix provided the best resolution and support from the singular gene analysis, revealing distinct separation between subfamilies and grouping of closely related species. Overall, the distribution of species across Miombo and Mopane ecoregions showed even representation across countries, with specific subfamily patterns. For instance, Cercidoideae was well-represented in both Miombo and Mopane, Detarioideae exhibited most species with habitat exclusivity, and the largest subfamily, Caesalpinioideae, demonstrated homogeneous distribution. The greater species diversity found in the Miombo woodlands is attributed to their larger area and more diverse environmental conditions.

Phylogenetic diversity and molecular data prove to be effective for understanding evolutionary patterns in tree legumes, underscoring the uniqueness of Miombo and Mopane regions. Further investigation into vegetation dynamics, anthropogenic and environmental drivers is urgently required, as well as the implementation of more conservation management strategies.

Presented by [Sílvia Catarino \(silvia.catarino@edu.ulisboa.pt\)](mailto:silvia.catarino@edu.ulisboa.pt)

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## Poster 9

### **Mutation Accumulation Experiments in *C. elegans* reveals genotype-specific dynamics associated with different population sizes**

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Mutation accumulation (MA) experiments are invaluable for unraveling fundamental aspects of evolutionary change, shedding light on mutation rates, their impact on fitness, and the intricate interplay among mutation, natural selection, and genetic drift. While MA experiments have been performed across a spectrum of organisms, including bacteria, fungi, fruit flies, nematodes, plants, and even human cell lines in vitro; they have not systematically characterized intra-specific variation in MA dynamics. To better understand variation of MA dynamics within the same species, in this work we established multiple *C. elegans* MA lines from five distinct genotypes, and performed MA during twenty generations while amplifying mutation rates up to 100 fold. This was done with populations maintained with either two or thirty individuals, facilitating the discernment of effects arising from the fixation of deleterious alleles with varying fitness coefficients. Fitness was measured for lines following the 20 generations of MA and after additional ten generations of inbreeding to estimate inbreeding load and purging levels. Our results broadly agree with expectations regarding accumulation of deleterious mutations at different population sizes, while underscoring the pivotal role of genotypic variation in shaping the fitness consequences of fixed mutations, particularly in influencing inbreeding load and purging dynamics.

Presented by Ivo Chelo ([immchelo@fc.ul.pt](mailto:immchelo@fc.ul.pt))

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## Poster 10

### Comparative demography of endangered species using genomic data

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Genomic data have become increasingly available for many taxa including endangered species. In addition, the computational methods used to reconstruct the demographic history of species from genomic data have become increasingly sophisticated. These developments in population genetics are very promising for conservation efforts in biodiversity hotspots, such as Madagascar. However, demographic studies still rely on simple models, often assuming panmixia thus ignoring the effects of space and population structure. To tackle these issues, novel methods have been developed to infer demographic parameters under various types of structured models. Here we focus on approaches based on the Inverse Instantaneous Coalescence Rate, IICR, as implemented in the Structured Non-stationary Inference Framework (SNIF) method and program. The main goal of this project was to use PSMC/IICR curves as input data and infer the parameters of piece-wise stationary n-island models using SNIF. We applied this approach to endangered species of Mouse Lemurs (*Microcebus* spp). To that end, we first used real genomes from single individuals aligned with the reference (high-quality) genome of a species from the same genus (*Microcebus murinus*) or with a de novo sequence from the same species. With this approach, we intended to study how trustworthy the PSMC plots are, to compare demographic histories of different alignments. We also used simulations to validate the scenarios inferred from real data. The usage of mathematical models and statistics applied to genomic data from a single individual allows us to study specific genomic signatures of coalescence and infer population structures, and thus explain demographic histories in light of paleoclimatic events. Better understanding the demographic history of endangered species enables conservationists to implement targeted and effective strategies to preserve and recover populations.

Presented by Miguel Cisneiros ([miguel.cisneiros@gmail.com](mailto:miguel.cisneiros@gmail.com)) *Eligible for best poster award*

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## Poster 11

### **The interplay between different types of negative interactions may generate priority effects between spider mite species**

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Given mounting evidence of their widespread occurrence across taxa, costly sexual interactions between species, termed reproductive interference, have recently garnered attention as an important determinant of species exclusion. These interactions generally hamper coexistence by generating positive frequency dependence, where species become more disadvantaged as their population declines. Although reproductive interference has been largely overlooked in the coexistence literature, a recent theoretical development integrated them into modern coexistence theory. This framework is commonly used to predict the outcome of competitive interactions between species; coexistence is possible when competition for food generates negative frequency dependence, such that rarer species have an advantage and are able to invade. The newly proposed formulation, however, is yet to be empirically tested. In this study, we used two closely related spider mite species to independently measure the impact of competition for food and of reproductive interference on each species' growth. We then applied the modern coexistence theory framework to predict the ecological outcome of these interactions when each type is acting alone and when both are present. We found that, in our system, the two types of interaction act in opposite directions and that both should lead to the exclusion of either species. However, when both are present, their joint action should generate positive frequency dependent dynamics, leading to priority effects: either species is able to exclude the other based on the initial conditions of the system. These results highlight the importance of considering sexual interactions in studies of species coexistence.

Presented by Miguel Cruz ([marcruz@fc.ul.pt](mailto:marcruz@fc.ul.pt)) *Eligible for best poster award*

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## Poster 12

### The role of chromosomal inversions in adaptation and speciation in *Littorina* marine snails

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Chromosomal rearrangements are known since the beginning of genetics. Inversions, in particular, have two peculiar evolutionary properties compared with SNPs that are likely to confer them a disproportionate role in adaptation and speciation: i) they modify the recombination landscape; and ii) they tend to have larger fitness effects due to their length. Although the study of chromosomal rearrangements was for a while limited to taxa in which chromosomes could be easily visualized, recent advances in genomics have enabled to start correcting this taxonomic bias. However, their evolutionary significance remains to be evaluated in many systems. Leveraging on our previous work on marine snails of the genus *Littorina*, which are increasingly recognised as outstanding models to study parallel evolution of ecotypes and speciation in the intertidal, we will present the first results of our research project aiming to understand the role of chromosomal inversions in adaptation and speciation in *Littorina saxatilis* in the Iberian Peninsula. By combining genomic and phenotypic data from multiple natural hybrid zones between the ‘crab’ and ‘wave’ ecotypes we identified multiple inversions of various size that seem to be involved in ecotype divergence and found that reproductive isolation between *L. saxatilis* ecotypes is stronger in Iberia than in Sweden, where similar studies have been conducted. Current efforts to compare the level of divergence across the same environmental axis between ecotypes and within ecotypes will be complemented by mating experiments to understand the contribution of inversions to the evolution of reproductive isolation from early to late stages of the speciation continuum.

Presented by Rui Faria (ruifaria@cibio.up.pt)

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## Poster 13

### DNA Barcoding and Phylogeny of *Miridae* Specimens from the Azores, mainland Portugal and Spain, Based on Mitochondrial COI Sequences

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*Dicyphine mirids* are zoophytophagous insects. As generalist predators, they are important biological control agents in protected crops, mainly species of the genera *Nesidiocorus*, *Macrolophus* and *Dicyphus*. From these, *N. tenuis* (Reuter) and several species of *Macrolophus* and *Dicyphus* have been recorded in the Iberian Peninsula. *M. pygmaeus* (Rambur) and *D. cerastii* Wagner have been recorded in the Azores but not *N. tenuis*. *M. pygmaeus* and *N. tenuis* are commercialized biocontrol agents. We aim to analyze the genetic diversity within Iberia and between Iberia and the Azorean populations of *M. pygmaeus* and *Dicyphus* sp. Genomic DNA was extracted from 38 specimens of *Macrolophus* sp., 61 specimens of *Dicyphus* sp. and a specimen of *Macrotylus* sp., for a total of 100 specimens. These were collected from multiple locations in the Azores (29 specimens), mainland Portugal (62 specimens), Spain (5 specimens), and from four different companies that produce and market invertebrate biological control organisms (4 specimens). DNA barcoding for all 100 specimens was done through PCR amplification and Sanger sequencing of a 650 bp fragment of the COI gene, using the LEPF and LEPR primers. Consensus sequences were blasted against the GenBank sequence database using NCBI's blastn suite. Identified specimens were assigned to the following species: *Dicyphus bolivarii* (n = 5), *D. cerastii* (n = 49), *D. escalerae* (n = 2), *D. pallicornis* (n = 4), *D. errans* (n = 1), *Macrolophus melanotoma* (n = 6), *M. pygmaeus* (n = 32), and *Macrotylus paykullii* (n = 1). An alignment of all COI sequences and GenBank sequences was generated, and phylogenetic trees were inferred with maximum likelihood and bayesian methods, using *Macrotylus paykullii* as an outgroup. Phylogenetic analyses showed that samples belonging to both genera form two fully supported clades. All five *Dicyphus* species were recovered with full support. All but one of the *Dicyphus cerastii* samples share the same haplotype, regardless of geographical origin. *Macrolophus pygmaeus* are recovered in four fully supported clades, which do not reflect their geographical origin. Specimens from mainland Portugal and Spain appear distributed in several clades. Commercial specimens, along with a Spanish specimen from Catalonia, form a single, fully supported clade. *M. pygmaeus* specimens from the Azores form a clade with other Portuguese specimens. *M. melanotoma* specimens, are returned in a fully supported clade. Our results highlight the usefulness of COI as a genetic marker to distinguish species of *Miridae*. Ongoing analyses of 22 samples of genera *Macrolophus* and *Dicyphus*, which were additionally sequenced with low coverage whole genome sequencing will hopefully provide insights into the population structure of *M. pygmaeus*.

Presented by Davide Galhofa ([dmgalhofa@fc.ul.pt](mailto:dmgalhofa@fc.ul.pt))

## Poster 14

### **ARIES - The variation of Iberian sheep under domestication: an archaeogenetics study**

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The earliest known sheep in Portugal are dated to c. 5450 cal BC, from the Neolithic site of Lameiras, near Sintra, and c. 5500-5250 BC, from Caldeirão cave, near Tomar. Zooarchaeological evidence indicates they were introduced by boat in the Early Neolithic. Also, genetic diversity of extant Iberian native breeds supports the influx of new stock from overseas. Osteometric studies of Iberian sheep pointed out 1) a change of body shape between Chalcolithic and Iron Age times; 2) a significant increase in size in the Islamic period, while the bone shape remained constant after Roman times. Moreover, written sources indicate that Romans improved the wool quality of their sheep in southern Spain. Therefore, several questions need to be answered to help us understand the evolutionary history of Iberian sheep from Neolithic to Modern times. Ancient DNA analyses are a powerful tool for reconstructing past events, however genetic data from zooarchaeological specimens collected from Iberian sites are scarce. In this project we propose a multidisciplinary approach, combining zooarchaeological and archaeogenomics data, which are critical for understanding both temporal and geographical dynamics that shaped the genomic composition of sheep in Iberia. It aims to elucidate the origins, evolutionary trajectories, and modes of improvement of Iberian sheep, covering key prehistorical and historical periods.

Presented by Daniel Gaspar (danibgaspar@gmail.com) *Eligible for best poster award*

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## Poster 15

### Colonisation and phylogeographic structure of *Oxycara* (Coleoptera: Tenebrionidae) in Boavista (Cabo Verde)

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*Oxycara* Solier, 1835, is a group of apterous tenebrionid beetles widespread in the Palearctic Region with some species in the Afrotropical and Oriental Region and which underwent radiation in the Cabo Verde archipelago, where 16 endemic species are present. Boavista, the most eastern island is inhabited by three of these endemic species: *O. boavistae*, *O. feae* and *O. gestroi*. Just 640 m southwest of the closest point to Boavista is the Ilhéu de Sal Rei. The diversity of insects on this islet is poorly unknown to date, but during field sampling, we found some specimens of *Oxycara*. To determine the origin of the Sal Rei population, phylogeographic studies of the three species of *Oxycara* of Boavista were carried out. In this way, we were able to determine whether colonisation occurred naturally or whether they were introduced from other areas not close to the Ilhéu Sal Rei. For this purpose, we analyzed their mitochondrial genetic diversity and structure. Results may guide conservation management by helping identify whether the population qualifies as an evolutionarily significant unit worth protecting or if it is indistinguishable from the widespread unit in Boavista.

Presented by Pilar Jurado-Angulo (pilarjurado@cibio.up.pt) *Eligible for best poster award*

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## MyxoHares: Unravelling the genomic landscape of myxomatosis susceptibility in Iberian hares

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The Iberian hare, *Lepus granatensis*, is a key prey species in Iberian ecosystems and an important game species in Portugal and Spain. In 2018 and 2019, outbreaks of myxomatosis in Iberian hares caused important mortalities, endangering the persistence of the species in the native ecosystems. This outbreak was caused by the host jump of the *Myxoma virus*, which is responsible for the disease in wild European rabbits. Understanding genetic susceptibility and resistance of the host hares to the virus is thus important to devise monitoring programmes that can contribute to control the spread of the disease and for the conservation of the species. It also provides a unique opportunity to explore the impact of emerging pathogens on naïve host populations using cutting edge genomic tools, the objectives of this research. We are developing a case study, under the Biodiversity Genomics Europe (BGE) project, focused on this biological system, which aims at demonstrating the power of genomic data, anchored on high quality reference genomes, to produce biodiversity applications. The reference genome of the Iberian hare was produced in the framework of a pilot project of the European Reference Genome Atlas (ERGA), a European network that aims to promote infrastructural and scientific collaboration across Europe to generate reference genomes. This project employs whole-genome resequencing data to decipher how genetic diversity of Iberian hare genetic has determined the capacity for resistance to myxomatosis. The study involves the analysis of whole genome sequences of pooled samples of *Myxoma virus*-killed hares and 100 hares that show antibodies (were infected) but did not develop the disease. Genome scans will be used identify the basis for resistance that can be incorporated in monitoring tools. Furthermore, analysis of the neighbouring species, *L. europaeus* and *L. castroviejoi*, unaffected by the myxomatosis outbreak, will further contribute to understand the architecture of resistance. Special attention will be given to candidate genes associated with myxomatosis resistance in European rabbits. The insights gained from this research are expected to guide informed conservation measures, offering stakeholders crucial tools to mitigate and control the spread of the virus in this ecologically relevant game species. This collaborative endeavour underscores the contribution of ERGA and BGE in advancing genomic research for biodiversity conservation and management.

Presented by João Pedro Marques (joao.marques@cibio.up.pt)

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## Poster 17

### Fostering scientific literacy at high school: a case study on the evolution of seasonal colour change

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Promoting science literacy among high school students is pivotal for fostering critical thinking and scientific knowledge, essential attributes for shaping informed and engaged citizens. Here, we showcase an annual project developed with a cohort of senior high school students for Biology class. This initiative, established through a partnership between a research centre and a local high school, allows students to experience a real research project, contributing to their understanding of scientific research and the scientific method. The implementation of the project followed a two-step approach: first, an introductory practical activity opened to the students' community, designed to recruit participants and introduce the research topic; then, weekly in-person sessions throughout a whole school semester dedicated to project development. Our project was focused on seasonal coat colour change, an adaptive trait of several species that allows adaptation to environments seasonally covered with snow but that is being challenged by climate change. This system served as a model to address key Evolutionary Biology concepts. Students were actively involved in all stages of the project, from data collection to the dissemination of results. Beyond contributing to their understanding of scientific research, this initiative allowed participants to develop their informatic skills, by using specialized data analysis software, and interpersonal skills, such as teamwork and communication. In an anonymous survey conducted after the conclusion of the project, 10 out of 11 participants stated that the experience significantly enhanced their understanding of the scientific research process. Our work exemplifies how science can be brought into a classroom and contribute to students' active learning and personal growth. Building on this successful initiative, we aim to introduce new experiences in similar contexts, ensuring a continued contribution to science education and fostering students' curiosity and critical thinking.

Presented by Inês Miranda ([inesmiranda@cibio.up.pt](mailto:inesmiranda@cibio.up.pt)) *Eligible for best poster award*

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## Evolutionary analyses: Phylogeny as a possible way to understand the trypanosomatid's main immune system scape mechanisms

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The kinetoplastida order covers a vast number of unicellular eukaryotes, many of which have adopted a parasitic lifestyle. Accordingly, it comprises protozoa belonging to the genera *Leishmania* and *Trypanosoma*, both with medical and veterinary importance and therefore impacting economic and public health. The difference between the genera can be established based on the morphological characteristics and parasite life cycle. However, phylogenetic classification requires the analysis of genetic markers that involve highly conserved gene sequences such as the small subunit of ribosomal RNA (5S rRNA), genes that encode glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) and spliced-leader RNA gene. The first two methodologies demonstrate the main subdivisions of the Trypanosomatidae family but do not allow the establishment of relationships between and intra-species that the last one makes possible. Besides, the evolutionary forces among genes encoding parasite virulence factors are poorly understood. In this spot, we have studied the identified kinetoplastid genes family, specifically, those that appear to mediate immune evasions such as major surface zinc metalloproteinase (MSP), superoxide dismutase, trypanothione reductase, cysteine proteinase, glycoconjugates, and lipophosphoglycan, complement regulatory protein (CRP), trans-sialidase superfamily, trypomastigote decay-accelerating factor (T-DAF), free glycoinositol phospholipids and mucin-associate surface proteins (MASPs), Arginase, Nitric oxide synthase, among others. Moreover, we analyzed the evolutionary history of genes related to the vertebrate host's immune response to these pathogens. Phylogenetic analyses were used to study the relationships between genes and genera. Therefore, a search was carried out and the sequences of interest homologous to *Leishmania* sp. and *Trypanosoma* genes were obtained in the database (NCBI and TritypDB). The DNA sequences were recovered using BLASTn and reference sequences characterized as a query, the trypanosomatids species data set was used to perform the searches. In addition, free-living *Bodo saltans* was used as an outgroup. The global alignment was performed using Clustal Omega. Thereafter, the MEGA Software was applied to generate a Maximum Likelihood phylogenetic tree (500 bootstraps). Also, a Bayesian inference analysis was conducted using BEAST2 to support and compare with the Maximum Likelihood trees. Our preliminary results showed gene diversification and expansion among trypanosomatids. However, it was not possible to obtain a tree with high support values for all nodes, mainly due to a high number of divergent sequences, sometimes partial and different in their domain. Trypanosomatid virulence mechanisms include complement system evasion as well as adhesion and host cell invasion. A detailed understanding of emerging aspects in the parasite's genome evolution will allow an integrated and targeted approach, without neglecting the discovery of drugs and vaccines, to improve control strategy.

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Presented by Cláudia Moreno ([moreno.claudia@ihmt.unl.pt](mailto:moreno.claudia@ihmt.unl.pt))

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## Poster 19

### Insights into the genomics of the Iberian dog breeds and searching for their influence on the development of Brazilian counterparts - preliminary results

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Dogs are an important genetic patrimony and in Portugal there are 11 functional autochthonous breeds, including local varieties within breeds. The analysis of their genomes can provide a good opportunity to guide conservation efforts in locally adapted dog breeds and understand the evolutionary processes behind their development. During the 16th to the 19th centuries, the migration routes established during the “Portuguese Maritime Expansion” between the Iberian Peninsula and the American continent via the Western coast of Africa brought “Old World” livestock to Brazil along with the dogs required for their management. Iberian dogs have gone through highly dynamic evolutionary processes, such as: their replacement or breeding with dogs from other regions; being shaped by natural and artificial selection; and the effects of isolation and genetic drift. The allele frequencies observed in extant dogs are the end product of these complex processes. For Portuguese dogs, we determined the phylogeny of maternal lineages, the nuclear markers genetic diversity within-breed and inferred the population structure. Our study included a total of 54 genomes, obtained from two Iberian wolves, 10 Portuguese and two Maghreb dog breeds. We analyzed these data in the context of 85 whole-genomes available from public databases: 83 dogs representative of 40 worldwide breeds, 1 Moroccan wolf (*Canis anthus*) and 1 coyote (*Canis latrans*). Our preliminary results have shown that: in general, Portuguese autochthonous breeds correspond to independent genetic units; their maternal lineages are distributed within major dog haplogroups A, B C and D; and some breeds require efforts for their long-term preservation. Ultimately, we aim to investigate the genomic composition and breed relationships of 20 dogs from 9 Brazilian breeds and landrace dogs, as well as 13 stray dogs from the island of S. Tomé and Príncipe. This should allow us to understand Portuguese and S. Tomé and Príncipe dogs’ legacy in the development of Brazilian dog breeds.

Presented by Ludmilla Paixão Blaschikoff (ludblaschikoff@gmail.com) *Eligible for best poster award*

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## Trust your guts? The effect of gut section on diet composition and impact of *Mus musculus* on islands using metabarcoding

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DNA metabarcoding is widely used to characterize the diet of species, and it becomes very relevant for biodiversity conservation, allowing the understanding of trophic chains and the impact of invasive species. The need for cost-effective biodiversity monitoring methods fostered advances in this technique. One question that arises is which sample type provides a better diet representation. Therefore, with this study, we intended to evaluate if there were differences in diet estimates according to the section of the gastrointestinal tract analysed and which section(s) provided the best diet representation. Additionally, we intended to infer the ecological/economic impacts of an invader as a model of the potential effects in an originally mammal-free ecosystem. We examined the gut contents of the house mouse *Mus musculus* introduced to Cabo Verde, considering three sections: stomach, small intestine, and large intestine. We applied a DNA-metabarcoding approach using two genetic markers, one specific for plants and another for invertebrates. We showed that this invader consumed 131 taxa (73 plants and 58 invertebrates). We obtained significant differences in the composition of two of the three sections, with a higher incidence of invertebrates in the stomach and plants in the intestines. This may be due to stomach inhibitors acting on plants and/or to faster absorption of soft-body invertebrates compared to the plant fibers in the intestines. We verified that the impact of this invader in the ecosystem is predominantly negative, as at least 50% of the ingested items were native, endemic, or economically important taxa, and only 19% of the diet items were exotics. Overall, results showed the need to analyse only two gastrointestinal tract sections to obtain robust diet data, increasing the cost-effectiveness of the method. Furthermore, by uncovering the native taxa most frequently preyed on by mice, this DNA-metabarcoding approach allowed us to evaluate efficiently which are at the highest risk.

Presented by Catarina de Jesus Pinho (cjpinho@cibio.up.pt) *Eligible for best poster award*

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## Poster 21

### Using genomics to dissect the underpinning of behavioural differences in mice

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The modern progress of genomic analyses provides unprecedented opportunities to investigate the genetic underpinning of important traits. Despite their similar morphology and genome, *Mus spicilegus* and *Mus macedonicus* have radically different behaviours. While *Mus spicilegus* individuals cooperate in building large mounds for storage and nesting, *Mus macedonicus* are solitary and aggressive. In this work, we will use whole genome sequencing (WGS) data to understand whether these marked behavioural differences are reflected and can be determined by differences in genetic variation along their genomes. Using these datasets, we will, for the first time using high throughput sequencing data, characterize the history of divergence between the species and quantify the magnitude of their genetic divergence, which previous studies suggest being very shallow. Exploratory genome scans will allow inspecting whether marked genetic differences between species can be detected, and whether the functions of such genes can be related with behavioural traits. In addition, particular attention will be given to candidate genes known to influence behavioural traits in other species. This work is part of an undergraduate internship project from the Faculty of Sciences of the University of Porto and may provide key insights onto the genetic determinants of different social behaviours in mice.

Presented by Rodrigo Pires (rodrigo.pires2003@gmail.com) *Eligible for best poster award*

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## Poster 22

### Phylogenetic Diversity to Unravel Evolutionary Patterns of Poales on Oceanic Islands

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Advancements in molecular techniques have widened the utility of phylogenetic diversity in biodiversity studies. This expansion positions phylogenetic diversity as a valuable tool for unravelling patterns and processes, especially in oceanic islands, which serve as natural laboratories for biogeographic and evolutionary studies. Despite the remarkable biodiversity of the Macaronesian Islands, there is a significant gap in our understanding of the phylogenetic diversity of key taxonomic groups, such as Poales. In this study, we conducted a comprehensive investigation into the phylogenetic diversity of Poales across the Azores, Madeira, Canary Islands and Cabo Verde. Our study provides unique insights into the evolutionary dynamics and ecological roles of this botanical order within the Macaronesian archipelagos. We obtained the phylogeny of ca. 475 Poales taxa in the Macaronesian region from a megaphylogeny for vascular plants based on molecular data. Our phylogenetic analyses revealed a tendency towards clustering in most archipelagos of the region. Species within the communities are more closely related than expected by chance, except for the Azores, where some islands display significant phylogenetic evenness. In addition, the distance between closely related species at shallower phylogenetic levels tended to decrease at higher latitudes. Most of the Cabo Verde islands exhibited significant phylogenetic clustering. Correlating phylogenetic diversity with environmental and climatic drivers could enrich our interpretation, enabling the testing of hypotheses related to species (co)evolution and maintenance mechanisms. The observed phylogenetic patterns carry implications for conservation strategies targeting the unique flora of this region. Integrating environmental factors into future investigations enhances our understanding of the mechanisms shaping biodiversity, facilitating sustainable conservation in these insular ecosystems. This study is supported by FCT/R&D Units Project Scope: UIDB/04129/2020 of LEAF and UIDB/00329/2020 of cE3c. Vanézia Rocha is supported by an FCT grant (SFRH/BD/151518/2021). We thank the financial support from FCT I.P. and the European Social Fund, through the Programa Operacional Regional do Norte (NORTE 2020), Programa Operacional Regional do Alentejo (Alentejo 2020), and the Centro Ciência LP.

Presented by Vanézia Rocha ([vanurocha2@gmail.com](mailto:vanurocha2@gmail.com)) *Eligible for best poster award*

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## Immunomodulatory effect of extracellular vesicles from *Trypanosoma cruzi*: an example of parasite-host co-evolution

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Parasites have a long co-evolution relation with their hosts, as they need to invade and subvert the host immune system to establish themselves. Consequently, *Trypanosoma cruzi*, the causative agent of Chagas Disease (CD), has evolved strategies to persist and disperse inside the host by evading, invading, subverting, and exhausting host immune defenses. This parasite can infect a wide variety of mammals including humans and domestic animals, in particular the dog, causing severe and life-threatening cardiopathies. In our globalized and fast-evolving world, CD has the potential to become a world health problem. The mechanisms used by *T. cruzi* for establishment in the host are still elusive and may contain the key to restraining parasite dissemination. As antigen-presenting cells (APCs), dendritic cells (DCs) are essential in the host immune response towards the parasite. However, little is known about the mechanisms used by *T. cruzi* to evade DCs activation. Also, in recent years, extracellular vesicles (EVs) naturally produced by all cells, have been attracting the attention of the scientific community as primary vehicles for transport molecular information. EVs are lipid membranes that can carry proteins and nucleic acids and exert immunomodulation on recipient cells. Therefore, this study aims to address the immunomodulatory potential of EVs naturally shed by *T. cruzi* on canine host DCs and in cardiomyopathy establishment. Canine monocyte-differentiated dendritic cells (moDCs) were cultured and exposed for 24 h to *T. cruzi* EVs previously isolated to allow EVs to interact with moDCs. In parallel, to address the immunomodulatory potential of parasitic EVs on the heart, cardiac tissue explant cultures were established and *T. cruzi* EVs were added. Toll-like receptors (TLR) and NOD innate immune receptors, as well as several key cytokines with pro- and anti-inflammatory activity were assessed by real-time PCR. The exposition to parasitic EVs revealed that moDCs and cardiac explants cultures were able to recognize and react to *T. cruzi* EVs by increasing the generation of TLR2, 4, and 9, as well as NOD2. The exposition to parasitic EVs also induced the generation of pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-12 that may contribute to the inflammation of the cardiac tissue associated with the establishment of severe cardiomyopathies. This response is balanced by the generation of IL-10 and TGF- $\beta$ . Therefore, parasitic EVs constitute an intrinsic part of the trypanosomatids biology and play a key role in orchestrating the parasite invasion and evasion of the host. This study illustrates one feature of the deep co-evolutionary relation between the *T. cruzi* parasite and the mammal host. Funding: This study was supported by FCT-Foundation for Science and Technology, I.P., through research grants EXPL/CVT-CVT/0175/2021 and PTDC/CVT-CVT/0228/2020, and by national funds within the scope of Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA, UIDB/00276/2020), AL4Animals (LA/P/0059/2020), Global Health and Tropical Medicine (GHTM, UID/04413/2020) and LA-REAL (LA/P/0117/2020).

Presented by Armanda Rodrigues ([armanda.rodrigues@ihmt.unl.pt](mailto:armanda.rodrigues@ihmt.unl.pt))



## Genome size variation and strategies to colonize insular habitats

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Relevant new cytogenomic data has shed some light on our understanding of evolution, but few studies were performed with insular and mainland populations, to test possible correlations with dispersal, speciation, and adaptations to insular environments. *Crithmum maritimum* (Apiaceae) is a monotypic genus that is distributed across the Mediterranean and northern Macaronesian islands and is a good model to study relationships between genome size variation and strategies of colonization of insular environments. Patterns of cytogenomic diversity were assessed among geographic samples (ca. 114) of *Crithmum maritimum*, collected across Azores and Madeira archipelagos, as well as in adjacent continental areas of Portugal. Using flow cytometry, the results indicated a significant intraspecific genome size variation, spanning from reduced sizes in the insular populations to larger ones in the mainland populations. Moreover, there was a tendency for an increase in genome size along the mainland populations, associated with lower temperatures, higher precipitation, and lower precipitation seasonality. However, this gradient might be the result of historic phylogeographical events associated with previous dispersal and extinction of local populations. Overall, our findings provided evidence that smaller genome sizes might play a critical role in the colonization of islands, corroborating other studies that argue that organisms with smaller genomes use fewer resources, having a selective advantage under insular environments. Although further studies are needed to improve our understanding of the mechanisms underlying genome size evolution on islands, conservation strategies must be promoted to protect the rich cytogenomic diversity found among *Crithmum maritimum* populations, which occurs in coastal areas that are particularly threatened by human activity, pollution, invasive species, and climate changes.

Presented by Maria Romeiras ([mmromeiras@isa.ulisboa.pt](mailto:mmromeiras@isa.ulisboa.pt))

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## Big and small: how do we fit all? Morphological evolution of sympatric and allopatric populations of small and giant Cabo Verde geckos

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Evolution can occur in sympatric and allopatric populations, i.e., populations that share or not the same geographic area, respectively. Arid islands are often great models for the study of competition in sympatric populations due as their scarce and limited resources. A possible consequence of sympatry is the enhancement of morphological differences between populations, i.e., character displacement (CD). Cabo Verde is an arid archipelago located in the Atlantic Ocean, and home to 12 *Tarentola* species, with most of them threatened but very little studied. These geckos arrived in Cabo Verde 7.7 million years ago from the Canary Islands in a single colonization event. From the same medium-sized ancestor, they developed into larger- (*Tarentola gigas*) and smaller-sized (*Tarentola raziana*) species. These two sister species were aympatric on Santa Luzia before the local extinction of *T. gigas*, and presently only on the islet of Raso. There, they are expected to compete with each other for the same scarce trophic resources. It was suggested the hypothesis of CD, but it was never explored in detail. In the present work, this hypothesis was tested using linear and geometric morphometrics on body size, head shape and size. Despite showing different conclusions, both approaches displayed significant differences among all populations, and between live and museum individuals for the same species. Besides uncovering a possible shrinkage in vouchers, a collector's bias towards larger animals was also proposed for the latter observation. The higher differences in sympatric populations may indicate some level of CD and ecological release in the allopatric population. Several alternatives for explaining these morphological differences are discussed.

Presented by Sofia Sousa (sofiacsousa.p6@gmail.com) *Eligible for best poster award*

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## Poster 26

### The impact of climate change on genetic exchanges across hybrid zones in hares

Sousa, P (1,2,3), Pimenta, J (1,2,3,4), Melo-Ferreira, J (1,2,3), Marques, JP (1,3)

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Climate change poses significant challenges to biodiversity, forcing species and natural populations to move, adapt or face extinction. Natural climate oscillations have historically influenced species ranges, leaving distinct genetic imprints that shaped their adaptability to changing environments. However, human-induced climate change has aggravated these challenges, leading to habitat fragmentation, population contractions of vulnerable species, and the expansion of favoured species. These contrasting demographic trends often result in species replacements in contact areas, potentially promoting hybridization and introgression when the species are not completely reproductively isolated. Understanding the influence of interspecific gene flow on the genetic variation of the affected species is therefore a crucial component of conservation strategies, as it can impact their genetic integrity by introducing deleterious or maladaptive variation. Nevertheless, interspecific gene flow can also introduce potentially adaptive genetic variation, and be a mean for species resilience by increasing their adaptive potential. Dissecting these genetic dynamics is essential to evaluate and forecast the influence of climate change on species dynamics, especially for invading and contracting species. In this study, we will investigate the genetic interactions between two species: the mountain hare (*Lepus timidus*), adapted to colder alpine-arctic climates, and the European hare (*Lepus europaeus*), a temperate species. Using RADseq (Restriction-site Associated DNA sequencing) data, we will explore the patterns of hybridization between these species. By characterizing these hybridization events, we aim to decipher the genetic exchanges that occur in species with contrasting demographic trends. This work is part of an undergraduate internship project from the Faculty of Sciences of the University of Porto, and will provide valuable insights into the mechanisms underlying species interactions in a context of climate change, informing conservation strategies and enhancing our understanding of current biodiversity dynamics.

Presented by Pedro Sousa ([pedrolucasassuncaoosousa@gmail.com](mailto:pedrolucasassuncaoosousa@gmail.com)) *Eligible for best poster award*

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## Poster 27

### **Flexible microbiome and plastic pace-of-life (POL) strategy among and within desert adapted species**

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How organisms adapt to their environment and tolerate change are key questions in evolutionary ecology. Pace-of-life syndrome (POLS) hypothesis predicts that species, populations, and individuals differ in a suite of co-evolving life-history, physiological, and behavioural traits, but co-evolution of these phenotypic components often varies along environmental/ecological selection gradients, and may limit, or be limited by, plasticity in heterogeneous environments. Furthermore, the gut microbiota influences, and is influenced by, host biology and can play a role in adaptation and plasticity, making it difficult to predict the conditions under which POLSs evolve. To improve our understanding of the processes of diversification and the origins of the unusually high endemism in the Sahara-Sahel, we are testing multiple hypotheses surrounding modes of adaptation and microhabitat specialisation. We will measure behaviour and metabolism at the species, population, and individual level and collect climatic, landscape, ecological, and microbiome data to determine ecological and phylogenetic patterns of POLS and whether trait variation in wild species and populations is due to consistent individual differences or plasticity. We will determine how climate affects spatial distributions of microbiota and POLS, and the potential of microbiota to influence host response to changing environments.

Presented by Alexandra Tyers ([alexandra.tyers@cibio.up.pt](mailto:alexandra.tyers@cibio.up.pt))

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## Fluctuations in migration rates and their impact on parapatric speciation

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Migration is of crucial importance in the broader study of evolution and, specifically, in the process of speciation. Migration will play a decisive role in deciding the future of incipient species (except when speciation occurs between completely isolated populations). However, most theoretical models assume that migration is constant over time, and the effect of fluctuating migration rates had never been investigated in the context of speciation. In this project, we use mathematical models to study how a time-dependent migration rate will impact the speciation process and the built-up of reproductive isolation between two populations. Using a two-locus continent-island model and Bateson-Dobzhansky-Muller incompatibilities, we investigate when reproductive isolation can start building-up on the island. We quantify how the fluctuations of the migration rate, both their amplitude and frequency impact the maintenance and stability of the system. Through numerical exploration of the system of differential equations, we showed that the stability of the system depends on the main mechanism of reproductive isolation and the frequency of oscillations of the migration rate: When selection against hybrids is the main mechanism, the dynamics of the system remains close to the expected equilibrium for the current migration rate. The strength of the genetic barrier depends either on the mean migration rate when the latter fluctuates rapidly, or on the maximum migration rate when migration rate fluctuates slowly. Finally, when the genetic barrier mainly depends on selection against immigrants, we observe the emergence of cycling dynamics, with the dynamics of the system departing from the expected equilibrium.

Presented by Joana Santos Belo ([joanacatarinasantosbelo@tecnico.ulisboa.pt](mailto:joanacatarinasantosbelo@tecnico.ulisboa.pt)) *Eligible for best poster award*

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## Molecular Phylogeny of Long-Eared Hedgehogs (*Hemiechinus auritus*) in Turkiye and Neighboring Areas

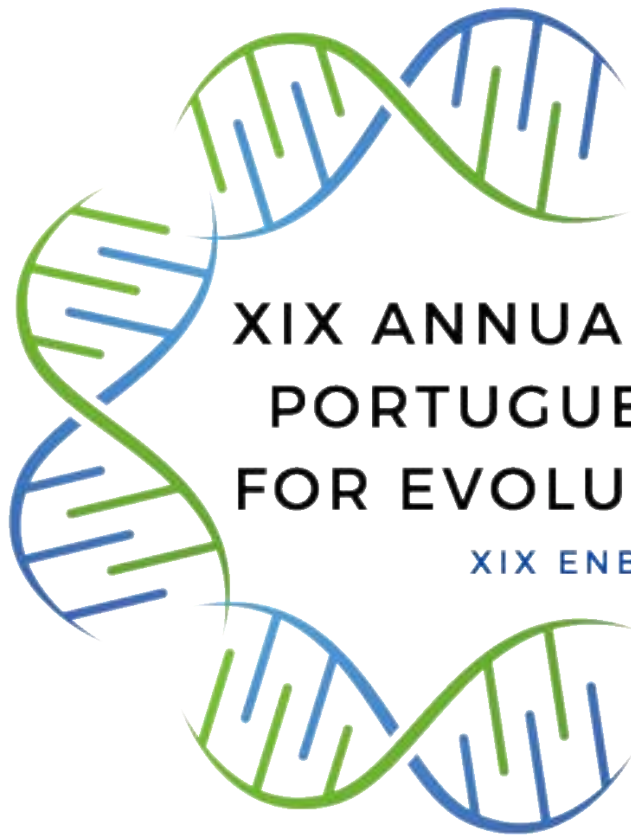
Özmen, M (1,2); Demirtaş, S (1); Herman, JS (3); Gündüz, İ (1)

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The long-eared hedgehog (*Hemiechinus auritus*) is one of the hedgehog species native to Central Asian countries including the some countries of the Middle East, with a range extending from the eastern Mediterranean region, through the arid and steppe areas of Asia to western Pakistan in the south, and from eastern Ukraine through Mongolia to China. Although this species occupies vast areas of the western Palearctic biogeographic region, its ecology and genetic properties are rarely documented, and few genetic studies that have been carried out so far are limited to populations in North Africa, Central Asia and Middle Asia. There is no information on the genetic diversity and structure of this species in Turkiye. Here, we use a mitochondrial DNA (cytochrome-b gene) sequences from specimens collected across the species' geographical range in Anatolia to provide insights into evolutionary genetics of this species. We also sequenced specimens from Cyprus and Azerbaijan to explore the systematic and phylogeny of the species. Cytochrome-b data confirm the existence of distinct eastern and south-eastern geographical lineages (differing by ~%3,15) in Anatolia, we consider them to represent two subspecies which are the most part separated territorially. *H. auritus* is extremely variable in the majority of the morphological traits studied. The number of subspecies is uncertain. By including published molecular data, we produced a well-resolved molecular phylogeny which is compatible with the morphology-based taxonomy, suggesting existence of all of the named subspecies listed by Hutterer (*H. a. auritus*, *H. a. albulus*, *H. a. megalotis*, *H. a. aegyptius*, *H. a. libycus*, and additionally *H. a. calligoni*), though more studies, based on morphological and other nuclear DNA markers data from more specimens across the species range, are necessary before any clear taxonomic conclusions can be drawn.

Presented by Medine Özmen (mmedineozmenn@gmail.com) *Eligible for best poster award*

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# XIX ANNUAL MEETING OF THE PORTUGUESE ASSOCIATION FOR EVOLUTIONARY BIOLOGY

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