



# Book of abstracts

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## Sponsorship

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Dear all,

Welcome to the National Museum of Natural Sciences (MNCN), the temple of Natural History in Spain! It is a pleasure to host you for the next few days and have the chance to reconnect after a long pause due to COVID19. You will find a very busy schedule of presentations (in various forms) as well as a nurtured programme for social activities. We wanted to give everybody a chance to speak during the conference and also an opportunity to talk and network in a more relaxed way. Truth is, we are very happy that so many of you wanted to come and engage in this event with us!

We are delighted to welcome you in this beautiful scenario, the MNCN, which recently celebrated its 250 anniversary, being as vibrant and dynamic as ever. It is an amazing setting that you sure have to take some time to explore, but it is also home to dedicated scientists working in biodiversity, evolutionary biology and ecology, global change, microbiology, anthropology, mineralogy and biochemistry.

We do not want to miss the opportunity of highlighting the incredible number of fantastic contributions we received, exploring amazing evolutionary histories, using the latest genomics techniques, and unravelling remarkable biological processes underlying ecological adaptations that are now threatened by global warming. We also wanted to dedicate some time to delve into how metabarcoding is revolutionizing the field of biodiversity exploration and include some insights into the use of artificial intelligence in our field.

Last but not least, we wanted to thank our superb panel of plenary speakers, who encompass brilliant scientist in three different stages of their career. Their contributions will surely entice your interest and hopefully inspire the new generations to dedicate their lives to this wonderful research field. Finally, a special word to our sponsors, without whom this conference could not have been realized.

This leaves us with nothing else than to wish you a fruitful conference and a great stay in Madrid.

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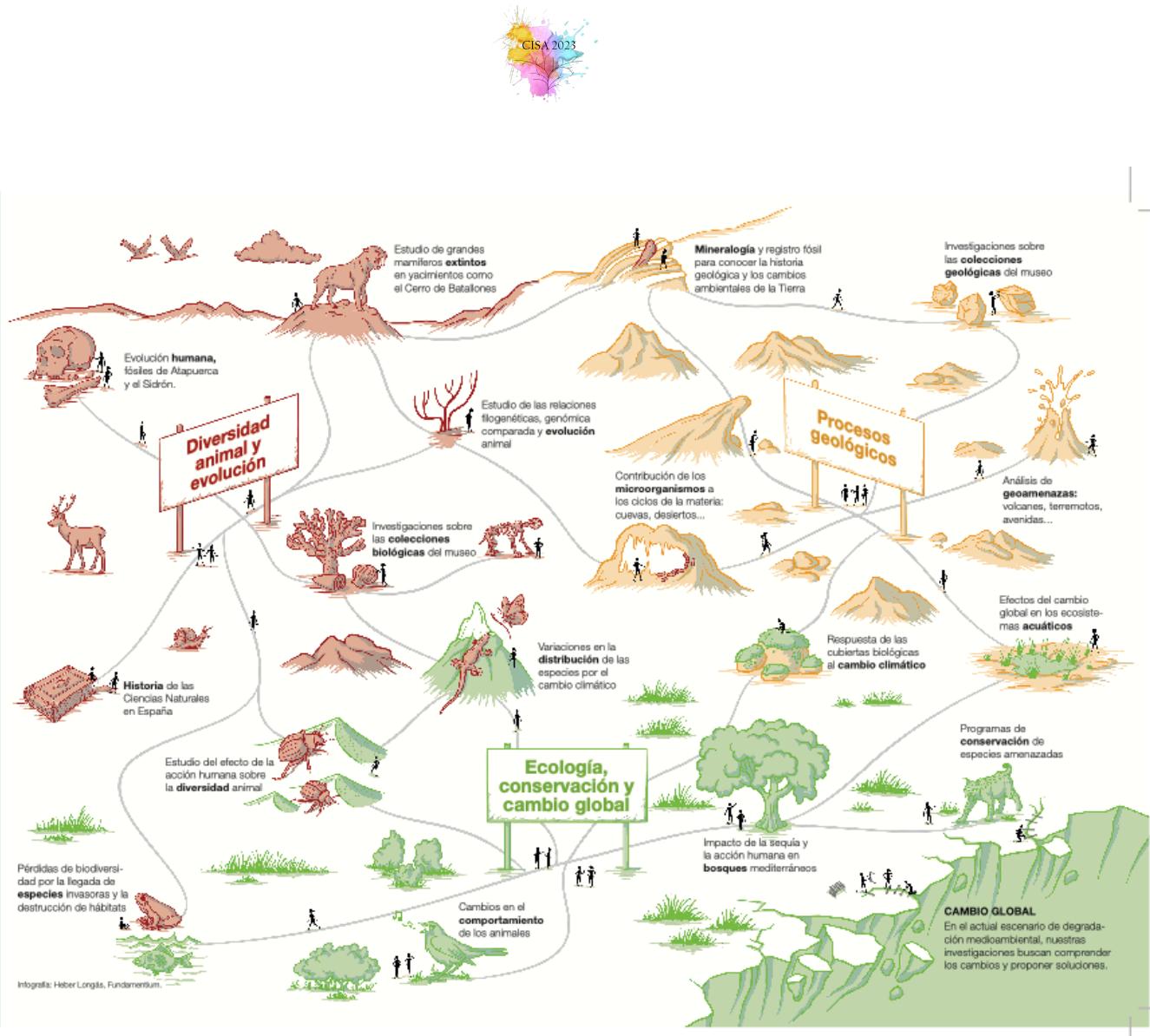
## The National Museum of Natural Sciences



The National Museum of Natural Sciences in Madrid is a treasure for lovers of nature and science. Founded in 1771 by King Charles III as the Royal Cabinet of Natural History, this museum is one of the most important scientific research and dissemination centers in Europe. With an extensive collection of more than eleven million specimens, the National

Museum of Natural Sciences is a fascinating journey through the biodiversity of our planet. The museum is divided into several sections, each focused on a specific branch of both biology and geology. Visitors can explore impressive exhibits showcasing a multitude of animals, minerals and fossils from different eras and parts of the world. These collections house a great diversity of species from various biogeographic regions, collected throughout scientific expeditions carried out by naturalists in the 18th and 19th centuries, as well as by researchers from the museum itself in more recent times, from every continent, including Antarctica. The National Museum of Natural Sciences is also dedicated to scientific research and the conservation of biodiversity. Its team of scientists actively works to expand knowledge in various areas, contributing to the understanding of the evolutionary mechanisms that generate and maintain biodiversity, as well as the geomorphological processes that shape the Earth's surface. This without forgetting the effects that climate change and human activity have on ecosystems, including losses of diversity, changes in the distribution and behavior of species, and alterations in biogeochemical cycles whose objective is to provide crucial information to make informed decisions and mitigate environmental impacts. In short, this museum is a window to the natural world that not only fascinates science enthusiasts, but also educates and inspires present and future generations about the importance of conservation and understanding of our planet. The National Museum of Natural Sciences in Madrid is a must-see destination for all those who wish to explore and appreciate the beauty and diversity of the natural world, and we truly hope you have the opportunity to explore and enjoy it during this conference.

Rafael Zardoya San Sebastián  
Director of the National Museum of Natural Sciences





# Plenary talks

## **Science (fiction) stories of hopeful monsters and genomic dark matter**

**Rosa Fernández**

Metazoa Phylogenomics Lab, Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra), Spain



We are living in an exciting era for the field of evolutionary biology. Recent methodologies, such as chromatin conformation capture techniques and artificial intelligence, can now be relatively easily applied to interrogate genome evolution in nonmodel organisms at an unprecedented level. We have reached a point where scientists can pursue ideas that only sounded like science fiction just a few years ago. In today's talk, I will share two stories about how we are leveraging these new tools to understand animal terrestrialization in my lab. The first story is about how drastic changes in genomic architecture, rather than punctual changes in the gene repertoire, triggered the origin of all terrestrial forms in one animal phylum. The second story involves applying natural language processing algorithms to infer the putative function of genes without homology across the Animal Tree of Life. I hope these stories serve as catalysts to spark discussions on the potential of these new methodologies to enhance our understanding of animal evolution to an unprecedented extent.

## The diversity, evolution and importance of protists

**Michelle M. Leger**



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Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

The vast majority of eukaryotic diversity is comprised of protists, a collective term for the primarily microbial eukaryotes that are neither animals, plants, nor fungi. Despite the taxonomic and morphological diversity of these organisms, and their ecological importance, they remain mostly poorly studied. I will argue that a greater appreciation of protist diversity allows us to more accurately understand the evolution and ecological context of the more familiar macroscopic eukaryotes.

The Opisthokonta include animals and fungi, as well as protists closely related to both lineages. Within this group, the protists most closely related to animals comprise members of four distinct groups that encompass a variety of morphologies, habitats and ecological niches. I will outline our current understanding of holozoan protist diversity and evolution, and I will discuss how a newly discovered holozoan parasite is helping us to clarify our understanding of early animal evolution, differing adaptations to parasitism, and taxonomic relationships within Opisthokonta.

## **Machine learning and new inference algorithms: expanding what is possible in evolutionary biology and phylogenetic analysis**

**Isabel Sanmartín**



Real Jardín Botánico (RJB-CSIC), Madrid, Spain

Investigators across different fields of evolutionary biology are increasingly turning their attention to more integrative and complex inference models. This is driven partly by the advent of next generation sequencing technologies, scaling up the numbers of genes, individuals and species sampled, and partly by the exponential growth of biodiversity and biogeographic databases at the local, regional and community scales. This massive increase in the quantity of data available has opened up a range of new questions but also unexpected challenges that require the development of more sophisticated approaches to data modeling. For example, a standing challenge is how to hybridize lineage-based, individual-based, and gene-based models to account for all axes of biodiversity loss (richness, abundance, diversity) in explaining the biotic impacts of climate change. The COVID pandemics introduced the need for models that are computationally efficient and can analyze in a short time thousands of sequences differing only in a few nucleotides. Statistical models in evolutionary biology are typically built around stochastic parameters linked by "hard-coded" dependencies, but there is an increasing demand for models that allow "hidden", unobserved components or that include a directional component (non-stationary, non-equilibrium models). Finally, many commonly used models of evolutionary inference have issues with non-identifiability: different model parameters induce the same probability distributions and are mathematically or "practically" undistinguishable. Phylogenetic inference itself is considered a non-polynomial time problem, meaning that the number of steps needed to solve a problem grow exponentially with the size of the problem (e.g., MCMC Inference). In my talk, I present possible solutions and paths forward in relation to these challenges, including the advent of online (Bayesian) phylogenetics, the development of machine (deep) learning approaches, and the introduction of probabilistic programming languages that promise to speed up analyses and tackle more complex biological questions in fields as diverse as phylogenetics, epidemiology, community ecology and phytogeography.



# Talks and speed talks

# Taxonomy & Systematics



## Taxonomy &amp; Systematics: Session T2: talk

**One genome to recover them all:  
The potential of low-coverage genome sequencing for phylogenetic  
inference and molecular evolution**

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High-throughput sequencing (HTS) has revolutionized phylogenomic and molecular evolution studies. Unlike Sanger sequencing, HTS allows the sequencing of millions of fragments simultaneously, dramatically increasing the amount of data obtained per run. The possibility of generating large datasets using a time and cost-efficient strategy provides a more thorough insight into biological evolution at multiple scales.

Low-coverage whole genome sequencing (lcWGS) has emerged as a popular HTS approach for the generation of phylogenomic data from model and non-model organisms. It is a cost-effective alternative to whole-genome sequencing, which requires less computing power and processing time. Moreover, unlike target capture HTS strategies, lcWGS allows the in-silico recovery of multiple types of loci, enabling the combination of newly generated data with legacy Sanger and target enrichment (e.g. UCE) information.

Here we use low coverage genomes, ranging from 1X to 10X, for assembling and annotating complete mitogenomes and recovering ultra-conserved elements (UCEs), universal single-copy orthologs (i.e. BUSCO genes) and household nuclear markers (18s, 28s, h3, h4 and its) from specimens belonging to the red devil spider family Dysderidae and allies. With the assembled data, we interrogate different aspects of the evolution of red devil spiders, ranging from species relationships to mitogenomic synteny analysis. We further investigate the effects of sequencing coverage on loci recovery success and demonstrate the potential of lcWGS as a cost-effective alternative for evolutionary research.

## Taxonomy &amp; Systematics: Session T2: speed talk

**Not one but two invasions of the Atlantic Ocean from the Indo-Pacific:  
Genetic and morphologic study of the slug genus *Vayssierea* Risbec, 1928  
(Mollusca, Nudibranchia)**Canet-Miralda, Carla<sup>1\*</sup> & Moles, Juan<sup>1,2</sup><sup>1</sup> Department of Evolutionary Biology, Ecology, and Environmental Sciences, Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain<sup>2</sup> Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Barcelona, Spain<sup>\*</sup>[ccanetmi10@alumnes.ub.edu](mailto:ccanetmi10@alumnes.ub.edu)

*Vayssierea* is an understudied nudibranch genus characterized by its orange coloration and small size of up to 5 mm long. There are four described species so far, distributed in the Indo-Pacific Ocean. Here, individuals of *Vayssierea* have been recorded for the first time in the North Atlantic Ocean, in the Canary Islands (Spain). This study aims to evaluate the systematic and taxonomy status, and distribution of the genus through a multilocus phylogenetic analysis.

Phylogenetic results show the monophyly of the genus, with a particularly long branch that may represent its fast evolutionary rate compared to their counterparts. Furthermore, the systematics of the genus is clarified, and we provide evidence that *Vayssierea* is included in the new subfamily Okadaïinae stat. nov. within Polyceridae. According to the species delimitation test, four different species have been sequenced from Russia to Australia in the Indo-Pacific plus our records from the Atlantic, but more information is needed to identify them. Nevertheless, our specimens from the Canary Islands correspond to two different species, one identical to the Australian species. Bearing in mind they lack a planktonic larval phase, we hypothesize that they have arrived by shipping transportation or aquarium releases, becoming an invasive species of the Atlantic Ocean.



Image 1: Photo of an individual of the genus *Vayssierea* from La Palma, Canary Islands

## Taxonomy &amp; Systematics: Session T2: speed talk

**Mallorcan anchialine caves provide new discoveries of enigmatic annelids, as well as insights on coastal cave adaptations and connectivity**Capa, María<sup>1\*</sup>, Pons, Joan<sup>2</sup>, Martínez Alejandro<sup>3</sup> & Martin Daniel<sup>4</sup><sup>1</sup> Department of Biology, University of the Balearic Islands, Spain,<sup>2</sup> Institut Mediterrani d'Estudis Avançats (IMEDEA-CSIC-UIB), Spain<sup>3</sup> Molecular Ecology Group, Water Research Institute (WRI-CNR), Italy<sup>4</sup> Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Spain<sup>\*</sup>[maria.capa@uib.es](mailto:maria.capa@uib.es)

Anchialine caves (marine caves with not obvious connections to the open sea) are singular environments characterised by lack of light, constant temperature, low nutrients and dissolved oxygen, and heavily horizontally stratified water layers. They harbour geographically restricted and ecologically specialized communities and consequently, they are considered unique systems of great conservation interest and highly sensitive to disturbance and. However, they are hardly accessible and, thus, one of the last unknown frontiers on Earth. Explorations of a cave in the north of Mallorca, Balearic Islands (Western Mediterranean) revealed the presence of very scarce but remarkable annelids, including two new species and a new record for the Balearic Islands. Some of these taxa show the typical morphological cave adaptations such as the reduction of eyes, elongation of sensory appendices and lack of pigmentation pattern.

In this study, we describe the cave environment and, thorough morphological and molecular analyses, we offer crucial information about the evolutionary relationships between cave and open sea taxa, while providing insights about potential connectivity and colonization patterns of cave environments.

Taxonomy & Systematics: Session T2: speed talk

## **Hidden diversity in a common Mediterranean marine annelid**

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*Amphiglena mediterranea* Leydig, 1851, was the only species in the genus reported in the Mediterranean Sea, for over a century. Recent studies in the Italian coast evidenced there are lineages compatible with the unified species concept hidden under an apparently similar morphological group.

In order to determine *Amphiglena* diversity along the Western Mediterranean and North-East Atlantic, an integrative approach for species delimitation combining genetic, morphological and also geographical data, was implemented. This represents the first integrative study in *Amphiglena* and the largest genetic study conducted within the genus.

The mitochondrial gene cytochrome C oxidase I, of 90 *Amphiglena* specimens, were analyzed. The genetic approach included phylogenetic analyses and different distance and tree based molecular species delimitation tools. The morphological approach included microscopic techniques.

Seven undescribed *Amphiglena* genetic lineages compatible with the unified species concept were found in this study adding to the other seven species already reported in the Italian coasts and Madeira. Western Mediterranean species showed restricted and, in some cases, sympatric distributions, indicating no genetic flow between taxa regardless proximity. Morphological features overlapped in certain molecular groups and five morphogroups were identified. *Amphiglena* holds a vast morphological and genetic diversity that has been overlooked over the years.

Taxonomy & Systematics: Session T2: speed talk

## **There and back again: A tale of the oak gall wasps' genus *Cynips* in the New World (Hymenoptera: Cynipidae: Cynipini)**

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Oak gall wasps belonging to the tribe Cynipini (Hymenoptera: Cynipidae) present a taxonomic challenge due to their complex biology. Many genera within this tribe have been defined by plesiomorphic character states, leading to polyphyly upon further examination. Synapomorphies are scarce among oak gall wasps, necessitating the use of non-exclusive character states to delineate most genera. Consequently, morphological resemblance may be observed among distantly related genera.

The taxonomy of the New World fauna has been additionally burdened by the strong tendency to homogenize the genus-level diversity between Europe and America. The adoption of several Palearctic genera into the species-rich Nearctic and Neotropical fauna has promoted the creation of those polyphyletic macrogenera. One of the best examples is *Cynips* Linnaeus, 1758 which was originally described in the Palearctic but rapidly gained representatives in the Nearctic with the first studies in the New World. Over the 20th century, several studies transferred all the species of *Cynips* into different genera, most of them newly described. The last efforts towards the unification of Palearctic and Nearctic diversity were the global revision of Melika & Abrahamson (2002) synonymizing 15 genera. Amongst the nomenclatural acts of this revision, *Antron* Kinsey, 1930, and *Besbicus* Kinsey, 1930 were sunk under *Cynips* Linnaeus, 1758, and hence, *Cynips* was re-introduced in the Nearctic after Weld's revision (1952).

The current revisionary process aiming to determine natural groups among the Cynipini genera has shed new light on the non-existence of *Cynips* in the New World. Molecular evidence suggests that *Antron* and *Besbicus* are independent of the Palearctic *Cynips*. Furthermore, the species originally included in *Antron* are nested in two independent clades which suggests the existence of a new genus. Given those results, Melika *et al.* (2021) re-established the genus *Antron*, and a thorough morphological analysis reveals solid



diagnostic characters to support the re-establishment of *Besbicus*, and the description of a new genus to the Nearctic fauna that includes species originally placed within *Antron*. A total of 53 species are transferred out of *Cynips*, 2 of which are placed within *Antron*, 8 within *Besbicus*, and 43 within the new genus.

## Taxonomy &amp; Systematics: Session T2: talk

**Unveiling hidden diversity: integrative species delimitation in the *Syllis prolifera* populations from the Mediterranean**

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Polychaete annelids play a dominant group within the marine benthic macrofauna, occurring in nearly all marine habitats from intertidal zones to deep sea, in both cold and tropical waters. Among them, the family Syllidae Grube, 1850 stands out as one of the largest and most diverse families of polychaetes, though they show a difficult taxonomy. Current taxonomic identifications often rely on morphologically ambiguous characters and frequently lead to misidentifications due to the presence of cryptic or pseudo-cryptic taxa. Additionally, molecular research has confirmed large distribution areas among annelids are scarce, and thus, ecological and molecular divergence without clear morphological differences, leads to cryptic or pseudo-cryptic speciation events, making it difficult to properly delimit species. *Syllis prolifera* Krohn, 1852, a putative cosmopolitan polychaete of the Syllidae family, inhabits a large range of different substrates usually across temperate and tropical coastal waters. In this study, we have used an integrative taxonomy approach to examine the morphological, ecological, and molecular features of populations from six geographic areas distributed across the Western Mediterranean Sea and the Strait of Gibraltar. Our multilocus molecular analysis including two mitochondrial DNA markers (COI and 16S rRNA) agree on the polyphyly of *S. prolifera*, indicating different levels of genetic differentiation between the *S. prolifera* populations sampled (48 specimens). The *S. prolifera* complex is distributed in two different well supported clades, nested with other *Syllis* species. Furthermore, species delimitation analysis identified at least five distinct lineages, some of which exhibited subtle morphological variations in the alternating length pattern of the dorsal cirri within the first five anterior segments and distinct ecological characteristics.

Overall, our findings do not support the cosmopolitanism, and not even the monophyly of *S. prolifera*, providing evidence of a new case of pseudo-cryptic speciation event within the family Syllinae, rather than a unique species with worldwide distribution. This study constitutes another example of the underestimation of the biodiversity of the group and highlights the importance of combined approaches to delimit species boundaries in polychaetes.

## Taxonomy & Systematics: Session T3: talk

### **Phylogenomics of chelicerate terrestrialization**

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The transition of life from the oceans to the land represented one of the most fundamental episodes in the evolution of life on Earth. Only a handful of animal phyla terrestrialized, such as vertebrates (once in tetrapods) and arthropods (independently in hexapods, myriapods and arachnids). Arachnids, a group of 12 terrestrial chelicerates (spiders, mites and related groups), were the first abundant animals in the terrestrial fossil record. They have been considered as a monophyletic group, which implies a unique and irreversible ancestral colonization of land. However, recent genomic-scale phylogenies do not support this relationship. Instead, they place horseshoe crabs (Xiphosura), a small group of marine chelicerates, within the terrestrial arachnids and not as their sister group. Under this scenario, the colonization of land by arachnids may have occurred on two or more occasions.

The aim of this work is to establish the interrelationships of chelicerates and their number of terrestrializations. These results will lead to a better understanding of the evolution of terrestrial adaptations (e.g. respiratory or sensory systems). Whole genome duplications have been identified in different chelicerate groups, making it difficult to distinguish between paralogs and orthologs, and leading to inaccuracies in phylogenetic inference. To minimize these errors and avoid hidden paralogies, we performed phylogenomic analyses based on very complete sets of genomic data, and using genes that are commonly found in single copy. We will discuss the challenges of resolving the evolutionary history of such an ancient animal group.

## Taxonomy & Systematics: Session T3: speed talk

### **Shedding light on the heterobranch's hidden diversity through worldwide sampling campaigns (Mollusca: Heterobranchia)**

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Since their rise around 400 Mya, heterobranchs embodied a diverse and charismatic group of marine, limnic, and terrestrial snails and slugs with a plethora of ecological and morphological adaptations to all environments. Altogether, heterobranch diversity encompasses ca. 44.000 living described species, but the cases of hidden diversity could greatly increase this sum. Here, we aim to shed light on the diversity of this group through worldwide collecting campaigns, developing different sampling techniques, and using molecular tools. Night diving, scraping algae, or extracting interstitial heterobranchs have been key in our field incursions to unravel hidden species worldwide. Among them, new species of nudibranchs and sacoglossans from the Maldives (Fig. 1 a-d) were described under the umbrella of "turbo-taxonomy", a methodology used here for the first time with molluscs. Special consideration is given to the mesopsammic groups, meaning those that inhabit the interstitial habitat. The restrictive ecological conditions of this neglected milieu, including faint light and a limited amount of space have forced its fauna, including heterobranchs as one of the most common groups, to adapt by simplifying their morphology. Thus, they are hard to collect and problematic to determine, often including cases of cryptic speciation as the ones revealed here for the nudibranch *Embletonia* (Fig. 1 e-f) or the 'lower heterobranch' *Rhodope* (Fig. 1 g-h), among others. The continuous findings of new species, commonly cryptic, highlight the need to gather more molecular and morphological data of this extremely diverse group.

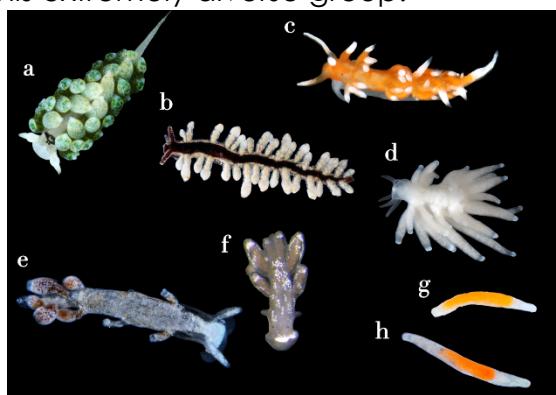


Figure 1. Live photographs of newly described heterobranch species, including (a) *Costasiella fridae*; (b) *Eubranchus putnami*; (c) *Sakuraeolis marhe*; (d) *Tergiposacca perspicua*; (e) *Embletonia* sp. 1; (f) *Embletonia* sp. 2; (g) *Rhodope* sp. 1; (h) *Rhodope veranii*.

Taxonomy & Systematics: Session T2: speed talk

### ***Alzoniella (Navarriella): A relict lineage of Hydrobiidae springsnails (Mollusca: Caenogastropoda) endemic to the Iberian Peninsula***

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The Iberian Peninsula harbours high numbers of endemic and threatened species of freshwater gastropods of the family Hydrobiidae W. Stimpson, 1865. Most of the 90 hydrobiid species described in the Iberian Peninsula are found in headwater springs and have poor dispersal abilities, restricting their distribution to a few isolated springs. In addition, their tiny size (shell size 0.5–5 mm) and simple morphology hamper the identification of hydrobiid species and their geographic limits.

Until recently, the systematics of hydrobiid species in the Iberian Peninsula was originally based on morphological characters, recognizing the occurrence of Mediterranean widespread genera in the region. Given that molecular data later classified some of these species into independent, narrow-range endemic genera, we aim to clarify the systematics and distribution of a widespread taxon with morphological species in the Iberian Peninsula, the genus *Alzoniella* Fo. Giusti & Bodon, 1984, using DNA-based methods. Specifically, we focus here on the subgenus *Alzoniella (Navarriella)* Boeters, 2000, which consists of two morphological species endemic to the northern Franco-Iberian mountains. Phylogenetic analyses based on two mitochondrial (mtCOI and 16S) and two nuclear (28S and H3) gene fragments classified the 46 studied specimens of both species (collected from 17 localities, including type localities) into one single species entity. Four molecular species delimitation methods (ABGD, bPTP, mPTP, ST-GMYC) confirmed this assignment. Within the Hydrobiidae, *Alzoniella (Navarriella)* was recovered as an old single lineage, distantly positioned from the type species of the nominal subgenus. These findings, along with the high genetic diversity and shell shape variation that we found in this hydrobiid lineage, highlight the role of the Iberian Peninsula as an evolutionary centre and refugium for springsnails and have important implications for the conservation of these imperilled taxa.

## Taxonomy &amp; Systematics: Session T1: talk

**Systematics of Iberian and northwestern African Akidini (Tenebrionidae: Coleoptera)**Jurado-Angulo, Pilar<sup>1,2,3\*</sup>, Recuero, Ernesto<sup>4</sup> & García-París, Mario<sup>1</sup>

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The tribe Akidini (Pimeliinae) consists of two genera in the western Mediterranean Region, *Akis* Herbst, 1799 and *Morica* Dejean, 1834, including a large number of local endemics on both sides of the Strait of Gibraltar. The recent succession of taxonomic proposals, considerably divergent and even opposed to each other, for the species of the genus *Akis*, highlight the taxonomic complexity of the group. This discussion is mostly a consequence of the difficulty of identifying specimens of related species because of the large phenotypic variability of their external morphological structures. Specifically, the most important contradictions arise in the interpretation of the elytral structure. Our main objective is to delimit evolutionary units within the genera *Akis* and *Morica* on both shores of the western Mediterranean and to discuss the variability and evolution of the external characters that were used to define taxa, on a palaeogeographic context. For this purpose, we elaborated a molecular phylogeny with mitochondrial and nuclear data, and performed subsequent morphological analyses. The study of the molecular and morphological patterns observed indicated that many of the species included in the most recent catalogs actually correspond to plastic phenotypes, often resulting from local hybridization.



## Taxonomy &amp; Systematics: Session T3: speed talk

**Taxonomic change and its impact on plant macroecology**

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Many aspects of macroecology depend on how we classify species. Although species taxonomy is an ever-evolving discipline, taxonomic change is rarely accounted for in macroecological studies. Here we elucidate how species description and subsequent taxonomic change impact documented morphological traits within species, range size, and geographic variation in species composition. We also tested whether species characteristics and the history of taxonomic discovery influence the likelihood of taxonomic change. We found that since the 1700s, nearly 1500 species names were condensed into the 187 accepted palm species. This reduction in the number of taxa triggered a cascading effect on documented trait variation within species, range size, and geographic pattern of species composition. Our study shows that taxonomic change is not random across taxa; taxa with large range sizes and those that were described in the early years of taxonomy are more likely to change. Our study highlights that key parameters used in plant macroecology are sensitive to change in species taxonomy, thus generating uncertainty in large-scale analyses and model forecasts. Based on these results, we argue that quantifying taxonomic change and their impact on ecological predictions continues to be a major challenge, whose resolution will ultimately require bridging taxonomy and macroecology.

Taxonomy & Systematics: Session T2: speed talk

**Systematic, biogeography and evolution of the *Lygodactylus gutturalis* (Bocage, 1873) complex.**

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Diurnal dwarf geckos of the genus *Lygodactylus* Gray 1864, are widely distributed in Madagascar and sub-Saharan Africa. *Lygodactylus gutturalis* represent a species complex which geographic distribution extends from Senegal in West Africa to Ethiopia and SW Somalia in East Africa, and to western Tanzania, southeastern Democratic Republic of Congo, and northern Angola in Central Africa. In this work, we use an integrative taxonomic approach for *L. gutturalis*, to unveil its complex systematics and evolutionary history. To this aim, we combined data from DNA sequences of mitochondrial and nuclear genes, external morphometric measurements, meristic characters including scale counts, high-resolution CT scans, and biogeography. Consequently, we shed light on its cryptic diversification and its evolutionary history within *L. gutturalis*, on which the recovered patterns of endemism from our combined analyses mirror those recovered in other groups of African squamates.

## Taxonomy & Systematics: Session T3: speed talk

### **Phylogenomic analyses identify Tardigrada as a member of Panarthropoda**

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Within the ecdysozoans, the phylogenetic position of tardigrades (water bears) has been particularly difficult to resolve. Even though morphological analyses repeatedly recovered tardigrades in alliance with arthropods and velvet worms (together called Panarthropoda), molecular-based phylogenies found them often as sister group to nematodes.

Here I use a novel dataset, as well as dataset specific substitution models to test hypotheses about the phylogenetic position of tardigrades within Ecdysozoa, in particular the alliance between tardigrades and nematodes. My results point towards a panarthropod nature of Tardigrada. Within this group, although not conclusive, my results favour either Tardigrada sister to Onychophora or sister to Lobopodia (Arthropoda + Onychophora).

Taxonomy & Systematics: Session T3: talk

## A resolved backbone topology for genus *Hypericum* based on phylogenomic data

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*Hypericum* L. ("St. John's wort") is in the top hundred largest angiosperm genera, and is composed of over five hundred species of herbs, shrubs, and trees, distributed worldwide, although they are mainly found in temperate regions of the Northern Hemisphere. In the last decade, several phylogenies, based on a few nuclear and plastid markers and comprising 20% of *Hypericum* species, have been published. However, these studies show neither support nor resolution for both the backbone and the most recent tips. Hyb-Seq, that is, target capture sequencing combined with genome-skimming, allows to obtain hundreds of low-copy nuclear orthologs and organellar DNA, needed to resolve species-rich genera, such as *Hypericum*.

Here, we implement a new specific customized probe set, the Clusioid626 kit, and show its effectiveness resolving phylogenetic relationships in *Hypericum*. In particular, we analyse ~260 samples of *Hypericum* (over half of the genus), belonging mainly to the so-called Old World group, as well as ten outgroups from the clusioid clade, to which the family Hypericaceae belongs. On average, our kit had a 66% enrichment success (measured as mapped reads on target) and resulted in ~500 single-copy genes.

Species trees were inferred using both ML and MSC approaches. The resulting topologies recover four out five formerly recognized clades, A through E, where clade C is instead a grade composed of three clades. Our species trees are mostly resolved and highly supported, both for the backbone and the most recent tips, and show that classical morphological sections do not represent the evolutionary history of this genus. In addition, we place, for the first time here, 190 species for which there was no sequence data available. A systematic revision, integrating morphological traits with our molecular data, is much needed.

Taxonomy & Systematics: Session T2: talk

## **Phylogenetic revision of Heterobranchia molluscs with 240 complete mitochondrial genomes**

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Heterobranchia is a diverse clade of charismatic taxa with many ecological adaptations to all environments, including nudibranchs, pulmonated land snails, the poorly known “lower heterobranchs”, among others. Their evolutionary relationships are still not well resolved thus requiring a significant systematic revision. In this study, we use mitochondrial genomes for resolving evolutionary relationships within this clade. These popular molecular markers are relatively easy to sequence, assemble, and annotate, and typically have a moderate level of sequence conservation that facilitates phylogenetic comparisons among relatively distantly related taxa. Also, gene order rearrangements are potentially phylogenetically informative. We have newly generated and annotated mitogenomes for more than 100 species of heterobranchs, including many key families, and combined them with all available data, having a total of 240 terminals. This is the largest phylogenetic analysis of Heterobranchia with mitogenomes that illuminates the backbone of their tree of life and provides interesting insights into their systematics.

## Taxonomy &amp; Systematics: Session T2: talk

**Exploring syllid annelids from Bermuda: an integrative approach unravelling the genus *Megasyllis***Moreno-Martín, Paula<sup>1\*</sup>, Verdes, Aida<sup>2</sup>, & Álvarez-Campos, Patricia<sup>1</sup>

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Syllinae is the most diverse and abundant subfamily within syllid annelids, but also the most problematic one in terms of its taxonomy and systematics. Several genera have been shown to be non-monophyletic in the most recent molecular studies and numerous morphological features traditionally used to identify species are homoplastic or ill-interpreted. In this study, we have carried out an exhaustive analysis of syllid species collected in Bermuda, combining both morphological and molecular data. Using optical and electron microscopy, together with the analysis of four molecular markers (cytochrome c oxidase subunit I, 18S rRNA, 16S rRNA and 28S rRNA), we have identified and established the phylogenetic position of 8 species (6 new) of some of the most problematic genera within Syllinae including *Haplosyllis*, *Opisthosyllis*, *Syllis* and *Megasyllis*, showing once again their non-monophyly. We place special emphasis on the genus *Megasyllis*, since is one of the most problematic genera within syllid annelid because the morphological characters traditionally used to recognize them, such as body shape, coloration, or type of chaetae, have proven to be homoplastic in the most recent molecular analyses. This genus is always recovered as paraphyletic in all previous phylogenetic studies, with *Alcyonosyllis* species nested within it and the genus *Paraopisthosyllis* as the sister group of both of them. *Megasyllis* and *Alcyonosyllis* mainly differ in the typical character used for species identification in syllids, the morphology of their chaetae, and they have therefore kept as independent genera. In the present study, we have compiled the largest molecular and morphological data set to date, including almost all currently known species, together with its closely related genera *Alcyonosyllis* and *Paraopisthosyllis*. In addition, we are including specimens of *Megasyllis* procera from Bermuda, being this the only species of the genus for the Atlantic Ocean, while the rest of *Megasyllis* species are restricted to the Indo-Pacific. We have performed a detailed morphological study of *Megasyllis* species and phylogenetic analyses using Maximum Likelihood and Bayesian Inference in 58 species of Syllinae, showing once again the paraphyly of the genus, in agreement with previous studies. Our results demonstrate the doubtful consideration of *Megasyllis* as a valid genus and we propose a taxonomical solution to resolve this issue.

Taxonomy & Systematics: Session T3: speed talk

### **Taxonomic and systematic updating of Iberian freshwater fish endemic species**

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The freshwater fish fauna of the Iberian Peninsula is characterized by a high level of endemism as a consequence of its old isolation from Europe (by Pyrenean Mountains) and from North Africa (by the Gibraltar Strait), being Iberian fish fauna isolated since at least five million years ago, in the border between the Miocene and the Pliocene. Despite this ancient origin, the number of Iberian species is relatively low, with approximately sixty-five described species. Most of the Iberian species have a restricted distribution range, sometimes circumscribed only to a few rivers, but unexpectedly even within this small occurrence area they usually have a high genetic structure, such as occur with *Achondrostoma salmantinum* or *Cobitis vettonica*. Conversely, some other species are extensively distributed throughout the Iberian Peninsula, as is the case of *Achondrostoma arcasii*, *Cobitis paludica* or *Squalius pyrenaicus*. Phylogenetic and phylogeographical approaches carried out on some of these widespread species have suggested the presence of undescribed taxa based on the identification of highly divergent genetic lineages and the genetic distances estimated between those divergent lineages. According to these findings, we have performed a deeper morphological and molecular study in three of the Iberian genera belonging to the families Cobitidae (*Cobitis*) and Leuciscidae (*Achondrostoma* and *Squalius*), leading to the formal description of several new species. In this talk we present the results from our recent investigations for these three genera in the Iberian Peninsula providing an updating of its freshwater fish fauna taxonomy.

Taxonomy & Systematics: Session T2: speed talk

## Disentangling recalcitrant phylogenetic relationships in Malpighiales with the Clusioid626 probe set

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The Clusioid Clade includes five families of angiosperm order Malpighiales, them being Bonnetiaceae, Calophyllaceae, Clusiaceae, Hypericaceae, and Podostemaceae, which comprise 94 genera and ~2.2K species, mostly found in the tropics. Order Malpighiales has been referred to as the most recalcitrant angiosperm group, since resolving phylogenetic relationships at various levels has proved to be quite the ordeal. Indeed, the Clusioid Clade, may not be monophyletic.

To address this challenge, we have designed a set of biotinylated probes, needed to implement a Hyb-Seq approach, which combines target capture sequencing (with streptavidin-coated magnetic particles) and genome skimming, and results in hundreds of low-copy nuclear orthologs, as well as organellar DNA (e.g., plastome).

The Clusioid626 kit was designed using, a reference genome (*Ricinus communis*) and transcriptomes for 12 species (with emphasis on genus *Hypericum*, the most species rich in the Clusioid Clade), belonging to five Malpighialean families. In total, the Clusioid626 kit is composed of 39.936 120-mer baits, for a total ~6.6M nucleotide data matrix, and mapping to 626 low-copy nuclear orthologs, including all Angiosperms353 targets, and that were selected taking their size and phyloinformativeness into account, to prevent methodological biases in the species tree inference.

We tested our probe set on 39 Clusioid Clade samples, and included data mined from the NCBI SRA for both 17 ingroup and 19 outgroup taxa. On average, 34.5% reads mapped to our targets (enrichment success), recovering ~600 orthologs. Following raw data quality controls, targets were assembled with HybPiper. Resulting data matrices were aligned with MAFFT and gene trees were computed with FastTree. A species tree under the Multispecies Coalescent model was inferred in ASTRAL. Our exploratory phylogenomic pipeline shows Podostemaceae may indeed not belong to the Clusioid Clade. Relationships for the remaining families are fully resolved and strongly supported, demonstrating the effectiveness of Hyb-Seq for recalcitrant plant lineages. Further, we infer Clusiaceae, rather than Bonnetiaceae, as sister to the remaining Clusioid Clade families. Similarly, we infer Calophyllaceae as sister to Hypericaceae, rather than Podostemaceae, which may be pointing to nuclear-plastid incongruence, as shown in previous studies, and which we will explore in the future.

Taxonomy & Systematics: Session T3: speed talk

## **Accounting for the taxonomic uncertainty in species occurrence records from biodiversity databases**

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In the last decade, biogeography and macroecology have made remarkable progress thanks to the information available in biodiversity databases. These databases are the result of a massive compendium of heterogeneous data from various sources. With such heterogeneity comes uncertainty in aspects like taxonomic identification, or temporal and spatial location of records of species occurrence, often compromising the reliability of the analyses conducted with these data. One of these sources of uncertainties corresponds to the taxonomic consistency and credibility of the records. For many groups, taxonomy can be controversial, with multiple databases and checklists published worldwide applying different criteria to classify species. This can create spurious biases that can affect the diversity patterns that are effectively observed. So to be comparable, records need to account for the dynamic nature of the scientific nomenclature and the differences between concurrent taxonomies. This poses a taxonomical challenge that should be overcome through the nomenclatural harmonization and standardization of occurrence records, a process that includes the selection of a taxonomical reference, the rank or taxonomic status considered or how to query names in databases. Here we present an example of how such heterogeneity in taxonomic information can be handled. For this, we use information on moss distribution in the temperate region of the Northern Hemisphere, a group of non-vascular plants that is estimated to have between 9,000 and 13,000 species. We evaluated whether the different checklists commonly used for standardizing species names may affect the observed spatial patterns of moss species richness. We used 3 different checklists of global flora 'The Plant List', 'World Flora Online' and 'Missouri Botanical Garden's Tropicos', and two general taxonomic databases that combine others, 'Taxonomic Name Resolution Service' and 'GBIF Backbone'. Our results show that the application of different taxonomies affects the overall number of currently accepted species and the observed species richness in cells of 100 x 100 km. Moreover, the choice of how to standardize species taxonomy can impact the geographical distribution of species richness. Due to the absence of a global authoritative reference of

moss taxonomy, we created a ‘consensus list’ of mosses that occur in the Northern Hemisphere. This consensus list includes accepted species names shared across the majority of taxonomic databases (three out of five in this study). This strategy is a conservative approach that may reduce the noise created by the taxonomical conflicts between checklists. Our analyses highlight the importance of carefully managing the taxonomic information in global scale studies. An inadequate handling of the taxonomy of occurrence records may add more noise and artefacts to the ecological and evolutionary inferences derived from them. On the contrary, a well-thought and careful processing and cleaning of data extracted from biodiversity databases can highlight disagreements and ambiguity in defining taxonomical concepts. This plays a crucial role in addressing issues of data quality in biogeography and macroecology, allowing the disciplines to identify potential errors that can affect the perceived diversity patterns of a group.

## Taxonomy &amp; Systematics: Session T2: talk

**From mitogenomic to multilocus, to transcriptomic, and to genomic: new resources that shed light on the phylogeny and evolution of caecilian amphibians**

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Caecilians (*Gymnophiona*) are, along with frogs and salamanders, one of the three orders of extant amphibians. They are a highly specialized group with elongate, annulated, limbless bodies, reduced visual systems, and with paired bilateral sensory tentacles on the snout. There are 221 extant species currently recognized, which are classified in 32 genera and 10 families, with mainly tropical distributions and burrowing habits. Most caecilians are terrestrial as adults, living in soil, but several species of one family (*Typhlonectidae*) are fully aquatic. Caecilians are an old group, with about 300 million years of separate evolution from their sister-group, the frogs and salamanders. Due to their specialized body form, ecological distinctiveness, and phylogenetic position in the vertebrate tree of life, caecilians are interesting for macro-evolutionary, life history, and evolutionary developmental biology research.

For nearly 20 years, we have been investigating the molecular phylogenetics and evolution of caecilian amphibians at a broad scale using mitogenomics (initially) and multilocus data. Our results have provided the largest, most inclusive and best resolved tree of life of caecilians thus far. Apart from state-of-the-art phylogenetic methods, we have employed information theory to elucidate the difficult branching order at the base of Teresomata (the largest and most diverse group of caecilians). Other highlights included the discovery of a new family of caecilians in India with ancient links to Africa. Generally, we have used our robust phylogenetic frameworks to study evolutionary patterns at different levels: molecular (mitochondrial gene order rearrangements), organismic (life-history evolution), and phylogeographic (ancestry and historical diversification).

In the last few years, we have also generated multi-tissue transcriptomic data for representatives of several caecilian families. This vast amount of information has been used to investigate the diversity of gene families in the context vertebrates, including the identification of homologous and orphan elements, annotation of unknown protein domains, and supertree phylogenomic analysis. Furthermore, we used the transcriptomic data to catalogue differentially expressed sequences (particularly in skin), and to identify genes under positive selection that could be associated to particular caecilian adaptations, such as fossoriality. More recently, three reference genomes have been generated for caecilians by some of our collaborators, and we are using this new information, along with the transcriptomic data available, to investigate the diversity and molecular evolution of defense bioactive elements of caecilians, in particular antimicrobial peptides.

Taxonomy & Systematics: Session T2: speed talk

### **Complex evolutionary history of *Liolaemus alticolor* (Iguania: Liolaemidae) revealed through phylogeographic analysis**

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The genus *Liolaemus* stands as one of the most diverse genera among South American lizards, with their evolution significantly shaped by the Andean uplift. While many taxa exhibit restricted distribution ranges, the phylogeographic patterns within most species remain enigmatic. *Liolaemus alticolor* offers an interesting case of study due to its extensive distribution, which extends across the Andean plateau from southern Peru and Bolivia to northern Chile. In this study, we uncover the phylogeographic pattern of this species across most of its distribution range, revealing a marked phylogeographic structure and the existence of a highly divergent "ghost" lineage. Additionally, the observed morphological variability does not totally align with the identified lineages. These findings suggest a complex evolutionary history of *L. alticolor*, marked by recurrent periods of range contraction and expansion along this Andean landscape.

## Taxonomy & Systematics: Session T1: talk

### **Systematic revision of the problematic sea slug genus *Doto* (Heterobranchia: Nudibranchia) – from the Mediterranean to South America**

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The genus *Doto* is probably one of the most taxonomically complex genera of nudibranchs due to its cryptic nature, the small body size, and very similar internal and external features. Here, a molecular revision of Mediterranean, South American, and Northeastern Atlantic species shed light on the controversial taxonomy of the group. The phylogenetic analyses and species delimitation tests show that coloration and external morphology seem to be unreliable characters to differentiate among many species. Interestingly, we found that the Mediterranean species described as *D. coronata* and *D. dunnei* correspond to a complex of three different species. The most common Mediterranean morphotype is *D. millbayana* (beforehand identified as *D. dunnei* and *D. coronata* found in seagrass meadows), and we confirm the presence of *D. coronata* and a new species of *Doto* from caves. A new record of *D. maculata* in the coastal western Mediterranean is also supported. In addition, several species from the Pacific Ocean are also unraveled. A detailed description of the external morphology and anatomy is given for the Mediterranean and South American new species. This integrative taxonomic description aids at the characterization and delineation of *Doto* species, suggesting possible evolutionary lines.



Figure 1. Live photographs several *Doto* species, including (A) *Doto maculata*; (B) *Doto coronata*; (C) *Doto eireana*; (D) *Doto verdicioi*; (E) *Doto pygmae*

# Barcode & Metabarcoding



## Barcode and Metabarcoding: Session B2: talk

**Metabarcoding, NUMTs, and taxonomic inflation: addressing a controllable challenge**

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Metabarcoding of Metazoa is increasingly being used to characterize large fractions of biodiversity, contributing to solving impediments associated with small-sized hyperdiverse invertebrates. Aligned with the standardization of the cox1 gene as the DNA barcode for metazoans, the use of cox1 gene fragments is gaining prominence in the field of metazoan metabarcoding. Its utilization offers notable advantages, ranging from the availability of extensive reference databases to the ability to detect spurious sequences that do not conform to the expected translation. Spurious sequences pose a general problem for metabarcoding approaches, as authentic amplicon sequence variants (ASVs) amplified from target genes are inevitably accompanied by non-authentic variants. The latter arise from errors accumulated during the amplification and sequencing steps, as well as from the co-amplification of gene duplicates and pseudogenes. This issue also affects metabarcoding using mitochondrial markers due to the co-amplification of the so-called nuclear mitochondrial sequences (NUMTs). In this talk, we will review the characteristics of NUMTs that make them a concern for metabarcoding, providing examples of their prevalence and the resulting taxonomic inflation effects. We show how the recently developed metaMATE framework allows for a comprehensive examination of multiple dimensions of abundance filtering and the evaluation of the prevalence of unwanted concomitant sequences. metaMATE facilitates decision-making regarding the required filtering stringency and can be employed to enhance the reliability of intraspecific genetic information derived from metabarcode data. The framework is implemented in the metaMATE software (<https://github.com/tjcreedy/metamate>). Ongoing developments on the metaMATE framework will be discussed.

## Barcode and Metabarcoding: Session B1: speed talk

### **Identifying the need of having molecular data to better characterize the biodiversity of the Balearic Islands**

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The historical production on biodiversity and species distribution, together with the explosion of genetic and genomic data in recent decades, can facilitate the identification of information gaps and research priorities in defined taxonomic groups and geographic areas. With the objective of performing such assessments for the Balearic archipelago, we have started by doing a systematic search of all records in the NCBI's database that include entries from samples originating from our area of interest. Additionally, we have taken the catalogue of species in the Red List for the Balearic Islands (plants and metazoans) and cross-referenced it with the NCBI's database to evaluate which species have genetic/genomic information and how much of this is coming from samples collected in the Balearic archipelago. Overall, our analyses have identified over 43.000 entries for the Balearic Islands in the NCBI's database, covering more than 1.300 unique taxa. The data is notably biased towards microorganisms (including virus and bacteria), with only over 10% of these entries corresponding to metazoans and plants. Concerning the species included in the Red List for the Balearic Islands, we have identified those species for which genetic or genomic information is publicly available. The limited number of records originated from samples collected in the Archipelago highlights the need for generating such type of data from local biological communities, aiming for a better characterization of the Balearic biodiversity and a set of tools useful for its conservation, management and biological/ecological research.

Our analyses have also identified the importance and the high degree of useful information that is lost for potential analysis due to metadata not being entered (correctly or at all) into public databases. There is a need for researchers to understand this missed opportunity and to devote time to this component of the research process.

## Barcode and Metabarcoding: Session B2: talk

### **Unravelling the hidden arthropod diversity in soils through wocDNA metabarcoding haplotype community data**

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Soil biodiversity plays a fundamental role in numerous ecological processes critical for the functioning of terrestrial ecosystems. However, our understanding of biodiversity has primarily relied on studies of aboveground organisms, leaving soil biodiversity largely unexplored and often considered a "black box" in the field of biology. This lack of knowledge hampers our ability to conserve the approximately 25% of Earth's total biodiversity that resides in the soil. Methodological and logistical limitations have hindered our comprehension of soil biodiversity, and this challenge is particularly pronounced when studying the significant and diverse arthropod mesofauna fraction of soil biodiversity. Fortunately, recent advancements in high-throughput sequencing technology have provided a tool to bridge this knowledge gap. In this presentation, I will describe how our team (the SoilEvoEco lab) utilises whole organism community DNA metabarcoding (wocDNA MBC) to quantify and understand the otherwise imperceptible arthropod megadiversity in soils. I will demonstrate how using species-level nucleotide variation within metabarcode data represents a significant advancement in the study of diversity patterns, enabling whole-community genetic analyses at a haplotype-level resolution. In this line, I will discuss our research on the major drivers of biodiversity generation, assembly, and persistence in continental and insular soils. By shedding light on the hidden world of soil biodiversity and unravelling its complex dynamics, our work aims to contribute to developing effective strategies for quantifying, conserving and managing this crucial component of Earth's ecosystems.

## Barcode and Metabarcoding: Session B1: speed talk

### **Novel metabarcoding protocol for Saprolegniales**

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In the last few years, there has been an increase in the number of studies using metabarcoding to assess diversity in ecosystems. The quality of this assessment depends on the taxonomical precision barcoding markers can achieve. Ideally, metabarcoding should rely on species level discrimination to achieve maximum accuracy, which implies using fast evolving marker genes.

Saprolegniales (Stramenopiles; Peronosporomycetes) are fungi-like protists and include about 20 genera and more than 100 species. Some of these organisms are well-known as pathogens for aquatic animals and plants threatening wildlife, aquaculture and crops. Most studies developed on these organisms are focused on particular species and rely on strain isolation. However, a general investigation of the communities can provide information on the distribution patterns of diversity and allow predicting zoonotic episodes.

In that purpose, we developed a metabarcoding protocol specific to Saprolegniales, ensuring fast, cost effective and reliable estimation of environmental diversity. We designed two primers that amplified specifically the 5.8S gene and the variable region ITS2 and of Saprolegniales. We tested their efficiency on 10 mock communities, 15 pure cultures, 10 water-filtered samples and 10 soil and sediment samples. In that purpose, we extracted DNA, amplified it specifically and sequenced each sample using Illumina Next Seq. We evaluated amplification biases in mock communities, and provided an evaluation of Saprolegnialean diversity in human impacted (aquaculture basins) and pristine sites. We propose this new protocol to be used to track emerging diseases and infection dynamics.

## Barcode and Metabarcoding: Session B1: speed talk

### **Rivers' connectivity studied through eDNA haplotypes. An early approach to infer intraspecific variation.**

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The field of Environmental DNA is experiencing a continuous development with the emergence of different applications. Main focuses of current eDNA research are on the use of molecular techniques in environmental samples to monitor biodiversity, detect scarce species and to be applied in different types of ecological assessment.

However, its use in population genetics is still challenging. In this study, metabarcoding analyses using COI (Cytochrome oxidase subunit 1) gene as genetic marker were employed in water samples to assess differences on intraspecific haplotypes within a fragmented river area.

Differences on haplotypes composition and richness was found depending on the selected species showing the importance of life history traits when their connectivity is compromised. Although it is still necessary to test and validate different strategies, the results obtained in this study offered an approach to infer intraspecific variation based on haplotypes obtained from environmental samples.

## Barcode and Metabarcoding: Session B2: talk

### Tales from the deep: using sponges to survey deep water ecosystems

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The deep sea is one of the least explored biomes, and there is an urgent need to investigate its biodiversity, given the recent interest in exploiting these resources and the effects of altering its conditions. Ongoing efforts include biodiversity surveys through trawls, video exploration through ROVs. Environmental DNA (eDNA) has proven itself a useful tool in this scenario - as hundreds of species can be detected simultaneously - although with the practical limitation of filtering large volumes of seawater to capture more species. To overcome this issue, we use natural sampler DNA: sponges filter hundreds if not thousands of liters of sea water per day, and accumulate the captured particles in their tissue. By sequencing DNA extracts from their tissues, we can obtain a more thorough vision of their surrounding ecosystem.

Here we present the biodiversity estimations of three deepsea biomes from the North Atlantic and Arctic Oceans using metabarcoding - with universal primers- of tissue samples from four Demospongiae species: the low microbial abundance (LMA) *Phakellia ventilabrum*; and three high microbial abundant (HMA) species *Geodia barretti*, *Geodia hentschelli* and *Geodia parva*. Our sequencing efforts reveal a huge diversity of fishes – 50 species - and benthic invertebrates from 12 phyla, thus allowing to characterize the diversity of these vulnerable assemblages. The diversity recovered per sample showcases the power of natural samplers to accumulate traces of many species' DNA as they filter volumes of water far outweighing the amounts feasibly processed directly on eDNA sampling.

The difference in performance between the four species was remarkable and could be a combination of the amount of microbiome carried by each host species; the differential affinity of the Leray primers with host COI gene or the relative proportion of host to nsDNA.

## Barcode and Metabarcoding: Session B1: talk

### **Worldwide diversity and geographic distribution patterns of water bears (Tardigrada) unveiled by high throughput sequencing**

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Tardigrades are a phylum of panarthropods widely known for their ability to withstand severe environmental conditions. A total of nearly 1500 species have been described to date, but there is still much unknown about their diversity, taxonomy, systematics and evolution.

Traditionally, it was assumed that most tardigrade species are cosmopolitan or have wide distribution ranges, freely dispersing around the globe and dwelling wherever the environmental conditions were suitable (i.e. they conform to the "Everything is Everywhere, but the environment selects"). However, this view has started to crumble recently with the emergence of molecular studies. Latest works focused on particular genera of tardigrades have unveiled a diversity of species higher than expected, each of them with a relatively reduced distribution area.

In this work, we carry out the largest sampling of tardigrade populations ever made, collecting thousands of individuals from different environments in all the continents. We sequenced a fragment of the mitochondrial COI marker from each specimen using high-throughput sequencing, separated them into MOTUs, and compared their distribution and phylogeographic patterns.

Our results reveal an astonishing number of tardigrade putative species, with a great majority of them with reduced geographic ranges. While some of the MOTUs have a relatively wider distribution, for example *Macrobiotus hufelandi* present across northern Europe, most of them are present in just a few or even a single locality. We detected only several cases of MOTUs located in more than one geographic area, but they may be the result of human-mediated translocation. Our analyses also hint at how past climatic or geological changes might have affected the current distribution of the most common MOTUs.

In conclusion, our work challenges the most prevalent ideas about the diversity and distribution patterns of tardigrades, providing a direct refutation of the "Everything is Everywhere" hypothesis, and opens a new field paradigm in the study of the systematics, taxonomy and population genetics of this enigmatic animal phylum.

## Barcodeing and Metabarcoding: Session B2: talk

### **Speeding up integrative taxonomy in the time of the biodiversity crises: the case of the red devil spiders of the Caucasus**

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The biodiversity crisis driven by human activity warrants the implementation of fast and integrative taxonomical approaches that secure species inventorying before extinction. Recent technological and methodological advancements, such as high throughput DNA sequencing and the use of hypervolumes to describe functional diversity, have the potential to help with this difficult undertaking.

The red devil spiders, family Dysderidae, are a highly diverse lineage of nocturnal ground dwelling spiders with a western palearctic distribution. Despite its high diversity and conspicuous morphology, the taxonomy of the family remains challenging, and some areas of its distribution poorly sampled. The dysderid fauna of the Caucasus provides a good example of these limitations. Here we conduct a taxonomic revision of the dysderid fauna of Georgia, the most diverse country in the region, based on recent collections.

To rapidly generate data for guiding taxonomic decisions, we combined a high-throughput sequencing DNA barcoding protocol, target gene sequencing and meristic morphometrics. With these data at hand, we aimed to provide an updated checklist, uncover the phylogenetic relationships among identified species, and characterize their morphospace using n-dimensional hypervolumes.

The barcoding protocol yielded positive results for 66% of the samples, a result which could likely be improved with a more specific primer choice for spiders. By applying single marker delimitation algorithms combined with morphological evidence, we recovered 26 species, 8 new, and 5 unconfirmed candidate species. Three different genera were identified, among which the species of the polyphyletic genus *Harpactea* Bristowe, 1939 in Georgia appeared close to genus *Dasumia* Thorell, 1875. The genus *Cryptoparachites* Dunin, 1992, was recovered as the sister group to the remaining members of subfamily Dysderinae, and the Georgian species currently assigned to

*Hygrocrates* Deeleman-Reinhold, 1988 most likely belong to it. The genus *Dysdera* Latreille, 1804 was the most species-rich group with 18 species. Our morphometric results revealed that *Harpactea* in Georgia occupies a narrower part of the morphospace, while *Dysdera* presents a much wider spreading. As already described in other species, we also found substantial differences in the shape of the mouthparts in *Dysdera* which most likely hints to different dietary specializations in the genus (e.g. oniscophagy).

## Barcode and Metabarcoding: Session B1: talk

### A DNA barcoding library for exploring the evolution and diversity of European ants

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Ants are the most species-rich and ecologically diverse group of social insects and are important components of ecosystems. Europe hosts approximately 620 ant species, and this number continues to grow each year as new species are discovered, and alien species are accidentally introduced.

Comprehensive sequencing of mtDNA and construction of DNA barcoding libraries provide useful tools for the detection of potential new species and invasive ones, as well as for studying ecological and evolutionary processes.

We assembled the first DNA barcoding reference library based on COI for European ant species, both native and alien, and we assessed their intraspecific variability. We obtained a 78.5% taxon coverage (489 species) and ~6,100 sequences (~3,700 newly sequenced and ~2,400 retrieved). The dataset covers 37 European countries and 61 islands.

We found striking differences across groups in terms of DNA barcoding performance. We also estimated the percentage of sequences with stop codons as a proxy of pseudogene incidence and compared it across genera. We produced species-level maps illustrating the genetic variability. We found high intraspecific genetic variability and, by applying different species delimitation methods, we found a large fraction of putative cryptic species. Genetic results allowed us to report new faunistic records both at the European and country levels. Remarkable phylogeographic intraspecific patterns in a number of species suggest the effect of glacial cycles in combination with a geographical framework in shaping the observed differentiation.

Keywords: cryptic diversity, phylogeography, Formicidae, Hymenoptera

## Barcode and Metabarcoding: Session B1: talk

### **Environmental DNA, metabarcoding & relatives**

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There has been an explosion of conservation and management studies that make use of environmental DNA (eDNA) to detect the presence of a species or characterise the community composition in a sensitive and cost-effective manner. Some studies went beyond detection and successfully assessed the biomass using targeted detection approaches, or evaluated the representation of certain species of the community using metabarcoding approaches. However, there are still very few (or no) studies making full use of the real advantage of environmental nucleic acids (eNA) (i.e. the non-invasiveness, the relatively lower cost and the ability to study taxa without observations and to span to larger study territories) to look at the physiology of certain species. After a decade of expansion and despite the eagerness of eDNA to serve as a remedy for all difficult questions in molecular ecology, it still does not fill some of the information gaps that trapping and capture provide.

Removing fish from the environment, although very insightful (can inform age, sex, developmental stage, diet, pathogens, health, hormonal levels, pollutants, etc), it is not only invasive or lethal but laborious and time consuming, and it only gives a snapshot of the studied parameters of a limited number of specimens. Studying species or populations with large distribution ranges can take time and resources and hence it can become prohibitively expensive while only providing information at a broad spatial grid resolution of one or a few species at a time. Here is where eDNA metabarcoding can more efficiently describe the community. Adding environmental RNA (eRNA) to the toolbox, in conjunction with methylation analysis, can potentially inform about age, sex, developmental stage, health, responses to stressors, pollutants and other biotic or abiotic factors.

Here we present first developmental methods and the prospects to unlock the capabilities of eNA and ultimately accomplish full potential of non-invasive monitoring with environmental samples with a focus on a fisheries setting.

## Barcode and Metabarcoding: Session B1: speed talk

**Towards a Canary Islands barcode database for soil biodiversity: revealing cryptic and unrecorded mite diversity within insular soils.**

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Soil arthropods diversity contributes a high proportion of the total biodiversity on Earth. However, most soil arthropods are still to be described, hindering our understanding of soil functioning and global biodiversity estimations. Inventorying soil arthropods using conventional taxonomical approaches is particularly difficult and costly due to the great species richness, abundance, and local-scale heterogeneity of mesofauna communities and the poor taxonomic background knowledge of most soil lineages. To alleviate this situation, we have designed and implemented a molecular barcoding framework adapted to soil fauna, which constitutes the basis for the Canary Islands Soil Biodiversity barcode database. This pipeline includes different steps, starting with a morphology-based selection of specimens which are imaged and DNA extracted non-destructively. Both images and voucher specimens are used to assign a taxonomic identification by morphological characters, that is checked for consistency with molecular information. Using this procedure, we have studied 239 mites from the Canary Islands, of which we recovered 168 barcode sequences, and that were morphologically identified as corresponding with 49 species, with 9 additional specimens that could only be identified at the genus or family levels. Multiple species delimitation analyses were run, including ASAP, bPTP, BINs, and 3% and 8% genetic distance thresholds, in order to compare molecular delimitations with morphological identifications. Additionally, a species-level search was carried out at the Biodiversity Databank of the Canary Islands (BIOTA; <https://www.biodiversidadcanarias.es/biota/>) to evaluate the number of species in our dataset that were not previously cited as present on the archipelago. In parallel, a sequence-level search of our sequences was performed against BOLD Systems. Our results reveal that multiple morphological species correspond to different molecular lineages, which points to significant levels of unknown cryptic diversity within the archipelago. In addition, we evidenced that multiple species in our dataset constituted new records for the Canary

Islands fauna and that the information for these lineages within on-line genetic repositories is very incomplete. Our study represents the first systematic effort to catalogue the soil arthropod mesofauna of the Canary Islands and establishes the scaffold for the Canary Islands Soil Biodiversity barcode database. This resource will constitute a step forward in the knowledge of these arthropods in a region of special interest.

## Barcode and Metabarcoding: Session B1: talk

### Contribution of vertical and horizontal transmission to microbiome composition through the ontogeny of the marine sponge *Crambe crambe*

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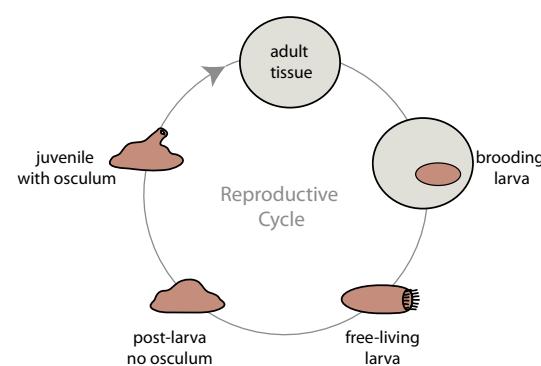
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Poriferans are highly adaptable organisms that can thrive in diverse marine and freshwater environments, thanks in part to their intricate associations with microbial communities. The sponge microbiome plays a vital role in facilitating beneficial biochemical processes for both the host and symbionts, exhibiting a high degree of specificity known as phylosymbiosis, which is specific to sponge species and individual genotypes. Sponges can acquire microbes from the surrounding environment (horizontal acquisition) and also transfer symbionts to their offspring through the sponge germline (vertical transfer), resulting in the presence of symbiotic microbes throughout all stages of sponge development, including adults, oocytes, embryos, larvae, and juveniles. However, the extent

to which the microbiome is transferred through each method remains poorly understood. Here, we assess the microbiome composition of a common and abundant Mediterranean sponge, *Crambe crambe*, across its ontogeny (Figure 1), including adult sponges, brooding larvae, free-swimming larvae, and settled larva or juveniles (without and with oscula). By analysing prokaryotic amplicons of 16S rRNA, we observed distinct microbiome compositions in each ontogenetic stage, characterized by



**Figure 1.** Schematic representation of *C. crambe* ontogenetic life cycle

vibrations in the presence of microbial species, as well as differences in abundance and diversity scores. Notably, a single dominant symbiont belonging to the Gammaproteobacteria class was consistently present throughout all ontogenetic stages, indicating its likely acquisition through vertical transmission from parents to offspring. The relative abundance of this symbiont fluctuated across developmental stages, with significant prominence during non-filtering states, where it accounted for over 70% of the microbiome's relative abundance. Both adults and juveniles with oscula exhibited the highest

microbiome diversity, likely attributable to their interaction with the surrounding environment through filter feeding, which facilitates the uptake of a broader range of microbial species via horizontal transmission. In contrast, brooding free-swimming and settled larvae displayed lower microbiome diversity compared to adults and juveniles, suggesting incomplete symbiont transfer to offspring or potential digestion of symbionts during non-feeding stages. Our work establishes the basis for future research to elucidate the dynamics of microbial composition during ontogeny, focusing on the symbiont acquisition strategies employed in each developmental stage and the potential roles of symbionts during larval development.

## Barcode and Metabarcoding: Session B1: talk

### **From metabarcoding to metaphylogeography: a protocol for the study of marine benthic communities**

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Metabarcoding is nowadays a recognized method for the assessment of biodiversity in many different habitats. This technique delivers, in a fast and cost-effective way, high amounts of biodiversity data, circumventing some of the problems associated with traditional sampling and taxonomic work. Metabarcoding, however, is not free from problems, and suitable protocols should be developed for each type of community studied.

Metabarcoding has a strong potential to be used, not only for alpha-biodiversity assessment, but also for biogeographic studies. In addition, metabarcoding databases can be mined for intra-species variation, allowing the simultaneous analysis of population genetic parameters of many species at a time, effectively opening the new field of metaphylogeography. Metabarcoding, therefore, provides an unparalleled opportunity to analyse the interplay of biogeography (at the inter-species level) and phylogeography (at the intra-species level).

We present here a complete protocol, from field techniques to bioinformatic pipelines, to analyse eukaryotic marine benthic communities using metabarcoding of the COI marker. We advocate direct sampling of the benthic community, as the use of eDNA from water did not provide an accurate picture of the benthos. Samples were obtained by conventional quadrat-scraping methods on rocky surfaces, followed by a size-fractionation step. We also propose a denoising software for the elimination of spurious sequences (generating correct Amplicon Sequence Variants, or ASVs) that takes into account the natural variability (measured as entropy) of each codon position in this protein-coding gene. Our protocol includes a clustering step using the SWARM procedure to obtain molecular operational taxonomic units (MOTUs) as a surrogate for species. Quality, chimera, and pseudo-gene filtering steps are also performed to generate the final database. All bioinformatic steps have been embedded in the user-customizable MJOLNIR3 pipeline. Community analyses and phylogeographic inference are performed with

standard multivariate and population genetic methods adapted to the nature of the metabarcoding datasets.

We present results obtained from shallow marine benthic communities from the Iberian Mediterranean littoral to illustrate the information that can be obtained with our protocol. We assessed the role that the hydrological discontinuities along the E Iberian coast (Almeria-Oran front and Ibiza Channel) play in shaping the alpha-diversity, beta-diversity, and phylogeographic structures of these communities in the area.

## Barcode and Metabarcoding: Session B1: talk

### **Novel biodiversity and multiple colonizations of athalassohaline water bodies by freshwater lobose testate amoeba (Arcellinida, Amoebozoa).**

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Arcellinida, or lobose testate amoeba, are a group of protists found mainly in freshwater and soil. They hadn't been recorded in marine environments, besides a few records at low salinity coastal lagoons. Until now, it was thought that Arcellinida had not crossed the salinity barrier. This barrier is considered as one of the most important barriers dividing biodiversity and strongly influencing the distribution of organisms. Crossing it requires profound physiological adaptations and is supposed to have happened rarely in the evolutionary history of the clades.

Most of these transitions have been studied in marine environments. Here, we explored the biodiversity of Arcellinida testate amoebae in athalassohaline water bodies (i.e. of non-marine origin and with different salt concentration and composition than the sea). These saline lakes experience extreme salinity and temperature fluctuations, which makes them arguably a harsher environment than the sea from the abiotic perspective. Biotic pressures, on the other hand, may be lower due to their island-analogous nature.

We combined microscopical observations, single-cell barcoding and environmental metabarcoding to explore the biodiversity of Arcellinida in different water bodies of Spain and Chile, with salinities ranging from freshwater to near saturation. We discovered a great amount of unknown biodiversity and multiple independent clades that had acquired the capacity to live in these saline environments, with various ranges of tolerance. Since Arcellinida are physiologically able to adapt to salinities even higher than those of seawater, salinity alone is not the barrier keeping Arcellinida from transitioning towards the marine environment.

This highlights how incomplete is the systematic and taxonomic knowledge of many groups of organisms, particularly protists, and the necessity of further sampling to complete these gaps of knowledge.

# Evolutionary Biology



## Evolutionary Biology: Session E2: speed talk

**Shedding light on the water-to-land transition of mudskippers**

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Among amphibious fishes, mudskippers (Gobioidei: Oxudercidae: Oxudercinae) are the ones that more outstandingly have achieved terrestrial adaptation. They live on mudflats and mangrove swamps of Africa and the Indo-Pacific region and are able to spend extended periods of activity out of water for some portion of their daily cycle (and even for some days). For this, they have evolved specific adaptations, including aerial respiration, ammonia tolerance, aerial vision, and terrestrial locomotion. Five out of the 10 oxudercine genera known are strictly considered mudskippers, and they have different degrees of adaptation to terrestrial conditions (including species that spend the majority of time out of water).

Over the last decade, we have assembled a multilocus dataset for a taxonomically dense sampling of all Oxudercinae genera, as well as representatives of closely related, mainly aquatic Amblyopinae and other gobioids. With this data, we have reconstructed a robust phylogeny and timetree that has been used to study biogeographical patterns and evolutionary trends in this interesting group.

Unlike the traditional view based on morphology, our multilocus results support that terrestriality has been achieved several times along the phylogeny of oxudercines and amblyopines, with completely aquatic (the majority) and amphibious species appearing interspersed in the tree. Our timetree indicates that this diversification occurred relatively recently, during the Oligocene, and it was likely associated to the historical biogeography of mangroves that favoured the recurrent and independent appearance of water-to-land transition scenarios for mudskippers.

More recently, we have examined genomic data for mudskippers and other amphibious fishes, in particular the molecular evolution and role of aquaporins (transmembrane proteins involved in osmoregulation) in their water-to-land transitions. Among other findings, we have found selective sequence changes in a particular key motif of one aquaporin class (AQP11b) that are possibly related to marine-to-freshwater adaptation in Gobioidei ancestors, and that could have been later exapted in mudskippers during their transition to terrestrial environments.

## Evolutionary Biology: Session E2: speed talk

### Unusual body pattern, reproduction and symbiotic way of life of branching worms

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Branching marine worms are annelids with one head but a body that branches over and over again into multiple posterior ends. Their unusual body pattern fits with the habitat in which they live: the labyrinthic internal canals of different sponges, with which they maintain a symbiotic relationship.

While there are more than 20,000 species of annelid worms, there are only three species with such a branching body pattern: *Syllis ramosa* McIntosh, 1879; *Ramisyllis multicaudata* Glasby et al., 2012 and *Ramisyllis kingghidorahi* Aguado et al., 2012. The biodiversity and systematics of these intriguing animals, since the discovery of the first described species in the XIX century found during the Challenger Expedition, until the description of the latest species dedicated to Gozillas' enemy will be explained.

Additionally, latest results about their evolution, reproductive mode and the symbiotic relationships with their host sponges will be presented.



Figure. *Ramisyllis kingghidorahi*, named after Godzilla's nemesis. Its head is at the bottom of the image. *Organisms Diversity & Evolution*, 2022. DOI: 10.1007/s13127-021-00538-4

## Evolutionary Biology: Session E1: talk

**The story of annelid regeneration and stem cell research through single-cell transcriptomics**

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Studies on cell identity and differentiation are nowadays carried out by using single-cell RNA sequencing (scRNA-seq), a powerful technology to simultaneously profile the transcriptome of thousands of individual cells. These analyses have already revealed the cell type atlas of several model organisms, opening questions about the molecular evolution of cell types and enabling the study of complex developmental processes such as regeneration. Regeneration, the ability to replace lost body parts, is a widespread phenomenon in the animal kingdom that has been studied by biologists for a long time in a wide range of animals including annelid worms. The freshwater species *Pristina leidyi* is one of the emergent annelid models due to its amazing regenerative capacities: it can regenerate both anterior and posterior fragments, starting from small pieces of tissue. Recent studies have morphologically and molecularly characterized the regeneration process of this species but the involvement of stem cells in it still remains a controversial question. Using our recently optimised pipeline for scRNA-seq, combining ACME dissociation with the cell barcoding protocol SPLIT-seq, we have generated the first single cell atlas of *P. leidyi* adults. In addition, we have also validated cellular identities through modern *in situ* hybridization experiments based on hybridization chain reaction (HCR) technology. Our results reveal the cell type diversity of adult annelids for the first time and serves as a resource for studying different developmental trajectories and the evolution of Annelida cell types.

## Evolutionary Biology: Session E3: talk

**Tempo and mode of diversification in red devil spiders (Araneae: Dysderidae) from the Canary Islands**

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Adaptive radiations have played a key role in our current understanding of species evolution, but few common underlying patterns have been found to describe this process. Previous studies examining the mode in which species diversified, how diversification rates shifted through time, and how ecological specialization influenced both aspects, have yielded mixed results. To gain further insights into the diversification process, additional model systems involving alternative ecological settings are needed.

Here, we present the results of a study aimed to unravel the diversification of the species rich genus *Dysdera*, the red devil spiders, in the Canary Islands. These species exhibit remarkable phenotypic variability in their mouthparts, which is related to different levels of trophic specialization to predate on isopods. Specifically, we used phylogenetic comparative methods to explore patterns of lineage diversification and assess the role of trophic specialization as a driver of species diversification. Additionally, we used climatic variables, occurrence data and geometric morphometric data to unravel the underlying mode of speciation by means of joint species distribution modelling and age-range correlation methods.

Our results show that Canarian red devil spiders underwent an early burst of diversification, followed by a slowdown of diversification rates, which is a hallmark of adaptive radiation processes. We also found evidence that trophic traits shaped diversification, with specialist species exhibiting higher rates of speciation. However, trophic traits may not have been the sole or primary driver of diversification. Finally, our analyses suggest that speciation occurred mostly in allopatry, with secondary sympatric distribution following range expansion.

## Evolutionary Biology: Session E2: talk

**A multiomics approach to understanding the colonization of land by free-living Platyhelminthes**

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The conquest of land constitutes one of the most important milestones in the evolution of life on Earth. Elucidating the genomic toolkit of terrestrialization is key to understanding the evolutionary processes that led to the diversification of life. In Platyhelminthes, contrary to other invertebrate groups, only one transition to land has been identified with confidence, with the family Geoplanidae (Tricladida) constituting the only true terrestrial platyhelminth lineage. This family includes a high number of large species and is particularly diversified in tropical and subtropical regions. The goal of this study is investigating the genetic underpinnings that facilitated the adaptation of planarians to terrestrial environments. To answer this question, we explored gene repertoire evolutionary dynamics in a dataset composed of 37 platyhelminthes including representatives of all main clades, with 15 newly generated transcriptomes from species of the family Geoplanidae. Our results showed higher rates of gene gain and duplication than loss, suggesting that an expansion of the gene repertoire was key in reshaping the gene repertoire of terrestrial planarians. Expanded gene families were enriched in functions related to cellular transport, DNA repair, UV response, negative phototaxis, regulation of metabolism and reproduction. New genes gained in land planarians were enriched in genes related to biosynthesis of sterols and respiration, indicating that genomic innovation took place in the terrestrial lineage in functions related to cell membrane composition and oxygen homeostasis. Our results set the road towards understanding the genomic basis facilitating the colonization of land in platyhelminthes at a deeper level.

## Evolutionary Biology: Session E3: talk

### **Patterns of diversification of a Neotropical crater lake cichlid fish**

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The formation of new species via ecological diversification is a major source of biodiversity. Populations diverge as a result of adaptations to alternative niches which involves changes to their morphology, physiology and behaviour, but also leaves traces in their genomes. In the early stages of divergence, genomic changes are restricted to regions encoding traits relevant for survival in the respective environment, while genomic incompatibilities may arise at later stages. Due to the rapid progression of speciation in adaptive radiations, genome-wide differentiation among species is generally low, but elevated in regions originally involved in divergence, which allows inference of the mechanisms that drive these radiations.

The Midas cichlid species complex (*Amphilophus citrinellus*) is native to the Nicaraguan great lakes region. It has independently colonized crater lakes from source populations of the great lakes. Following colonization, it has undergone independent and parallel adaptive radiations in the crater lakes, forming species flocks that have specialized on exploiting different niches. The Midas cichlid radiations in the various crater lakes are at different stages of speciation. In some, different morphotypes are recognized as independent species, while in others, they are considered polymorphic populations. Here we focus on the less studied colonization of the very isolated Crater Lake Asososca León.

The Midas cichlid population of Cater Lake Asososca León is at a very early stage of speciation. Two clearly distinct forms can be found in the lake that differ in morphology and physiology. So far, these forms are considered a single polymorphic population with unknown levels of genomic divergence. Here, we determine genome-wide differentiation between the two morphotypes and we identify regions of divergence and functional processes that are important during the first stages of speciation in this system.

## Evolutionary Biology: Session E2: speed talk

### **Ecological niche and spatial evolution of two Balearic endemic lizards under potential climate change scenarios.**

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Mediterranean islands are priceless biodiversity hotspots and host a high number of endemisms. Identifying and understanding how endemism biogeography will change under the potential future climate scenarios is a prerogative in ecology given the vulnerability of organisms with restricted geographic distributions. *Podarcis lilfordi* (Günther, 1874) and *Podarcis pityusensis* (Boscá, 1883) are two emblematic endemic lizards of the Balearic Islands. Historically, natural barriers have delimited their distribution and their biogeography is linked to the island's geological evolution. Indeed, the geographic range of the species *P. lilfordi* is restricted to the Gymnesian islands (Mallorca, Menorca, and nearby small islets), whereas *P. pityusensis* is only found on the Pityuses islands (Ibiza, Formentera, and nearby small islets). Although the current geographical range is well known for both species, their ecological niche, and the evolution of their distribution under different potential climate change scenarios have not been explored. To fill this gap, we used publicly available occurrences in GBIF and a hypervolume approach to define the multi-dimensional space of environmental variables that best define the climatic preference of these species. We first calculated the current realized niche and habitat suitability for both species in the Balearic archipelago. Then we projected the model outcomes to future climate scenarios considering one Global Circulation Model (MIROC6), two moderate development paths of society (SSP 245 and 370), and two time periods (2021-2040 and 2041-2060). Our projected ecological niches are in line with the current knowledge about the ecology of the species, since we broadly obtained different climatic niches for each species. Smaller and partially shared niches were probably due to misidentification of the species in the occurrence dataset. About the current habitat suitability, we identified different geographic ranges for the two species with an overlap on the south coast of Mallorca. Concerning future projections, the distribution of the species doesn't change compared with the current period. We consider that the lack of future variation of the habitat suitability can be due mainly to three factors: 1) The physiology of these organisms (terrestrial vertebrate ectotherm organisms are more sensitive to climate cooling than warming). 2) the strong mitigating effect of the sea on the climate variations for small islands. 3) the low temperature rise predicted by the Global Circulation Models chosen for our analyses. Although the habitat suitability of both lizards is not susceptible to climatic variation in the next 40 years, other factors, such as the change in land use and the introduction of alien predators, can have a stronger effect on the fitness and survival of these species.

## Evolutionary Biology: Session E1: speed talk

### **Posterior specification and cellular reprogramming of planarians: mechanisms of anteroposterior regeneration**

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Freshwater platyhelminths, commonly known as planarians, are an ideal model for investigating pattern and growth in the context of a whole and complex organism, owing to their remarkable capacity to regenerating any body part, including the brain, from small fragments. The basis of their unusual plasticity is the presence of a population of adult pluripotent stem cells, called neoblasts, which can give rise to any type of planarian cell. Recent studies have demonstrated that not only the presence of neoblasts is required, but also the constitutive activation of cellular communication pathways to initiate planarian regeneration and tissue renewal. All of these processes are encompassed within organizing regions or organizers, which activate signals that initiate cell proliferation and ensure proper specification. These regions constitute cellular domains that govern the pattern and growth of the surrounding tissue.

These organizers are essential for understanding the properties of anteroposterior (AP) growth in planarians. Recent studies have provided evidence that the Wnt/β-catenin signaling pathway serves as the primary mechanism responsible for the specification of the anteroposterior (AP) axis and the functioning of the A and P organizers. Organizer A is characterized by the expression of notum (a Wnt inhibitor) in specific muscle cells of the anterior midline, while Organizer P is characterized by the expression of wnt1 in a small number of muscle cells in the posterior midline. Based on the properties of these organizers, inhibition of notum during bipolar regeneration (a trunk fragment that must regenerate both the head and tail) results in "two-tailed" planarians, whereas inhibition of wnt1 leads to the formation of "two-headed" planarians.

Based on this signaling pathway, the combination of ATAC-seq and ChIP-seq analyses, integrating these results with RNA-seq data from wnt1 RNAi animals (organisms with wnt1 inhibition), has revealed cis-regulatory elements (CREs) (promoters and enhancers), as well as transcription factors (TFs) that could be implicated in the specification of Organizer P.

Among these candidates, EGRII, Post2c, Post2d, Lox5a, Lox5b, Nkx1, Nkx6.1, and Nkx6.2 have been identified as potential key factors required for the correct expression of wnt1 during the early stages of regeneration (0, 12, and 24 hours post-amputation).

## Evolutionary Biology: Session E1: speed talk

### **Comparative genomics of cone snails is ready to go: the chromosome level genome of the Canary Island cone snail, *Kalloconus canariensis***

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Cone snails (Caenogastropoda: Conidae) produce very complex venom cocktails, made up of short peptides called conotoxins, which interfere with the neuromuscular receptors of their prey (other snails, worms, or even fish). There are more than 900 described species of cone snails, and we are determining the highly variable composition of the venoms of the different species, combining genomic, transcriptomic and proteomic (i.e., venomic) analyses. To date, only two high-quality genomes of cone snails are available, despite their importance for understanding the evolutionary mechanisms underlying the origin and diversification of the venoms. Here, the high-quality genome of *Kalloconus canariensis*, a vermicivorous cone endemic to the Canary Islands, is presented, and a comparative analysis of this genome with the other two available cone genomes (*Dendroconus betulinus* and *Lautoconus ventricosus*) is performed for the first time.



## Evolutionary Biology: Session E1: speed talk

### **Understanding mud dragon (Kinorhyncha) growth through an integrative developmental study**

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Kinorhyncha, commonly known as mud dragons, is an enigmatic phylum of marine, microscopic organisms that thrive in diverse habitats worldwide ranging from intertidal to hadal depths. Kinorhynchs are part of the 'molting animals' known as Ecdysozoa and are closely related to loriciferans and priapulids forming the Scalidophora clade.

The kinorhynch body plan consists of a radially symmetrical retractable head, a neck, and a limbless trunk with distinct segmentation. Mud dragons are direct developers, lacking larval stages and hatching from the egg almost as a smaller version of the adult. The first juvenile stage (J1) hatches with a well-developed head, a neck, and at least 8 out of the 11 final trunk segments and goes through five additional stages (J2-J6) before becoming an adult. During post-embryonic development the trunk segments are sequentially added in a subcaudal region by an initial elongation of the terminal segment followed by a transverse division.

The available data on post-embryonic development in kinorhynchs is limited to studies mostly based on external observations using light- and scanning electron microscopy, while the molecular mechanisms of basic developmental processes are completely unknown. Here we present new insights into kinorhynch post-embryonic development based on live observations, DIC-LM, SEM and CLSM data, combined with cell proliferation and differential gene expression analyses in *Setaphyes kielensis*.

## Evolutionary Biology: Session E1: talk

### **Evolution of sexual reproduction in sponges: genotypic sex determination in eight species**

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Sex is a prevalent biological feature that is advantageous at many evolutionary levels. The determination of sex in animals can be either genetic (GSD) or environmental (ESD), with a panoply of different solutions in both cases across metazoans, including interesting newly discovered GSD mechanisms in cnidarians. Because sexual reproduction is so diverse and plastic in sponges, classical theory assumed that sex is not genetically determined, but environmentally, most likely using temperature cues acting upon the specific expression of certain genes. Here, we used RADseq and RNAseq sequencing in previously sexed individuals of several species of gonochoristic sponges to investigate sex specific loci present in one sex and absent in the other, as well as their expression patterns across sexes. We identified markers significantly associated to sex in all species, most showing differential expression related to sex, some with specific isoforms expressed in one sex or the other. Although most markers were sponge-specific genes with unknown function, some others had correspondence to annotated genes in the genome and transcriptome, including genes known to be related to sex determination in other species and are affecting their expression.

## Evolutionary Biology: Session E1: speed talk

### A genomic approach to the venomous horned vipers of the genus *Cerastes*

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The desert horned vipers of the genus *Cerastes* are a small clade of venomous snakes within the family Viperidae. According to previous morphological and molecular studies, the group is comprised by four species: two phylogenetically sister taxa, *Cerastes cerastes* and *Cerastes gasperettii*, with generalist habitat requirements and allopatric distribution across the Saharan and Arabian deserts; a more distantly related species, *Cerastes vipera*, inhabiting the sandy environments of the Sahara desert, and the enigmatic *Cerastes boehmei*, only known from a single captive specimen allegedly captured in Central Tunisia in 1991.

Here, we used long-read sequencing (HiFi reads, 30x) together with linkage (Hi-C, 60x) and Illumina (30x) data to assemble a reference genome for the Arabian horned viper (*C. gasperettii*). We also annotated the genome with a combination of extrinsic evidence (RNA-seq, Iso-seq and protein databases) and previous published reference genomes, placing special focus on the identification of venom toxins. Moreover, we sequenced whole-genome sequencing (WGS) data for more than 20 individuals including specimens from the three species (*C. gasperettii*, *C. cerastes* and *C. vipera*).

We report a chromosome-level reference genome for *C. gasperettii*, with a total of 19 highly-contiguous scaffolds (contig N50 of 45.6 Mbp), and we identified the toxins specifically expressed in the venom gland. Both population genomics and phylogenomics suggested a new phylogenomic relationship,

matching previous results using ddRAD data. The three species showed different evolutionary history patterns as well as differing heterozygosity levels. Overall, our study represents the first attempt to characterize, from a genomic perspective, the venom composition of a venomous snake species from Arabia.

## Evolutionary Biology: Session E2: speed talk

### **Exploring the interactions between emerging fungal-like pathogens and Artrophoda**

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The Saprolegniales (Oomycetes) are aquatic organisms that includes some emerging pathogenic species. These species were considered opportunistic pathogens, however, recent studies have shown that only species of a particular clade of this group, named “parasitic clade” show host preference to certain host (e.g., *Saprolegnia parasitica* in salmonid adults; or *S. australis* and *S. diclina* in embryonic stages of freshwater animals). Still, the preference to other freshwater organisms and the role as vectors of other organisms, such as the arthropods, has been little investigated. Thus, the aim our study was to explore whether parasitic Saprolegniales can colonize other aquatic organisms, such as Artrophoda and be dispersed by them. For this purpose, we have established an experimental infection-response model using the species system *S. parasitica*-Hydrachnidia. We selected species of the group Hydrachnidia (Acari, Parasitengona) since they provide several experimental advantages, such as: (i) they inhabit all known habitats in continental aquatic ecosystems, (ii) their larvae are dispersed by other arthropods, (iii) they are easy to transport, maintain and infect under laboratory conditions. Our results are allowing us to improve our understanding of key aspects regarding the interaction of parasitic Saprolegniales with aquatic animals to better understand their host-parasite interaction preference and, eventually, the evolution of the parasitic mode of life in the Saprolegniales.

## Evolutionary Biology: Session E1: speed talk

### **Deterioro de líquenes epífitos y sus microalgas simbiontes en la comunidad valenciana: técnicas de biomonitorización y genómica aplicadas a la conservación de la biodiversidad**

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Los líquenes son indicadores únicos del Cambio Global. Por ello, el estudio de la diversidad de líquenes epífitos ha sido ampliamente utilizado para monitorear los efectos de la contaminación del aire en bosques de la península ibérica. En la década de los 90 se establecieron estaciones de muestreo en el norte de Castellón y Teruel (Els Ports y Maestrazgo) y se realizó una evaluación del estado de las comunidades de líquenes calculando el Índice de Pureza Atmosférica (IPA). Además, se registraron síntomas de daño visible y parámetros de fluorescencia para varias especies de macrolíquenes. Así, se estableció una red de biomonitorización en el área dels Ports, que se revisó en 2018. Actualmente, se está desarrollando un proyecto que considera estos sistemas forestales, y otros en la Comunidad Valenciana, para examinar el efecto de los cambios ambientales en la diversidad, función y estructura de los organismos en los microbiomas del talo del liquen, casi 30 años más tarde. Se re-evalúan 7 estaciones dentro de esta red para analizar el estado de los bosques. Para ello, se han seleccionado 9 especies de líquenes epífitos y se han calculado dos parámetros: el índice IPA, y la cuantificación de daños visibles. Además, se han identificado el micobionte y su alga simbionte principal, para conocer la estructura de la comunidad de líquenes de las diferentes estaciones. Posteriormente, se utilizarán técnicas de metagenómica para determinar los cambios en la abundancia y la estructura de las comunidades de hongos, bacterias y algas desarrollándose en el talo del liquen, para determinar si existen diferencias en el holobioma de talos de líquenes dañados versus líquenes sanos.

PROJECTS: PROMETEO/2021/005 (GVA a EB) and PID2021-127087NB-I00 (MICINN to IGB).

## Evolutionary Biology: Session E1: speed talk

### **Molecular and evolutionary mechanisms related to parthenogenesis in oligochaetes**

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Parthenogenesis is a very common phenomenon in the animal kingdom and in most animal groups there are parthenogenetic forms. Although studies of isolated cases of parthenogenesis in groups of normally sexual animals can contribute to our understanding of the evolution of reproductive systems, studies in organisms where parthenogenesis has occurred independently in a large number of species may provide a better approach. This is the case with earthworms, which are considered hermaphrodites with cross-fertilisation, a mechanism that is the most common one in this group. However, this way to reproduce is not the only one, since nowadays, numerous parthenogenetic species are continuously discovered, most of them polyploidic. Additionally, there are some species that maintain both modes of reproduction. This makes oligochaetes excellent candidates for studying the molecular and evolutionary mechanisms associated with these striking ways to reproduce.

Because parthenogenetic lineages can arise from sexual species in a variety of ways, including bacterial infection, spontaneous loss of sex due to mutations in genes related to mating or in genes involved in sexual forms, contagion, or hybridization between individuals of the same or closely related species, our project aims to study the evolutionary mechanisms underlying the process using three different approaches. The first one is to examine the transcriptional changes that occur in individuals of two earthworm species, one sexual (*Eiseniella andaluciana*) and one parthenogenetic (*E. tetraedra*). This will allow us to understand whether the molecular toolkits are the same in both mechanisms and what are the molecular differences to trigger each of them.

In addition, and since the gut microbiome has been extensively studied in earthworms, the second objective involves its study and the possible relationship between the differences observed in the two modes of reproduction. The last goal is to establish the general cell atlas of individuals of the two species, in order to determine the different cell types and the differences and similarities in their expression in both types of reproduction.

## Evolutionary Biology: Session E1: talk

### **Consequences of asexuality on phylogenetic inference: ancient fissiparity causing topology shifts in planarians of genus *Dugesia*.**

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*Dugesia* (Tricladida: Platyhelminthes) is a diverse genus of freshwater planarians with a broad distribution. In the western Mediterranean several studies have tried to reconstruct the evolutionary history of the species of the genus to tackle the factors that have driven it. Morphological studies are limited in these animals because of asexual populations, that lack reproductive structures and, therefore, cannot be properly assigned to a species. Thus, molecular analyses were applied to address these issues. The focus of the present study is the group formed by *D. etrusca*, *D. liguriensis*, and *D. ilvana*. These species were considered endemic respectively from the Tuscany, Liguria, and the Elba island, where exclusively sexual populations were present; however, new populations belonging to the two first species were found in the Catalonian region of the Iberian Peninsula and in the Southeast France, some of them being fissiparous populations (asexual reproduction by fission).

In order to reconstruct the phylogenetic relationships of these populations, we obtained transcriptomic data from 3 fissiparous and 7 sexual populations of *D. etrusca* and *D. liguriensis*, as well as a sexual population of *D. ilvana*. Our results showed a rather strange but highly supported ladder-like branching pattern, in which each sexual population is monophyletic, while fissiparous individuals constitute each an independent branch, forming a paraphyletic group that includes the sexual populations of *D. etrusca* and *D. liguriensis*. Also, *D. ilvana* came as the sister species of the group *etrusca-liguriensis*. However, when we removed the fissiparous populations from the dataset, *D. ilvana* was placed as the sister species of *D. etrusca*, with *D. liguriensis* as their sister species. Our hypothesis for this placement change of *D. ilvana* is that the presence of highly divergent alleles in the fissiparous individuals causes an artefact in the phylogenetic reconstruction, thus modifying the topology of the trees. This is supported by the unique ladder-like pattern present in the fissiparous

populations, showing these highly divergent alleles at intra- and inter-individual levels (i. e. Meselson effect), which point to an ancient age for the fissiparity in these populations.

Here, we present a phylogeographic scenario for the group, taking into account how the inclusion in the analyses of the fissiparous populations causes *D. ilvana* to change places in different analyses. To support our hypothesis of ancient fissiparity as the cause of the shifting, we will also present evidence that the ladder-like topology is an effect of this ancient fissiparity, and not an artefact of the orthologous search strategy or the phylogenetic inference method. We have run multiple analyses including different strategies of orthologous search, inclusion of paralogs, and different inference methods. Finally, we have obtained and annotated chromosome level genomes of sexual individuals from both *D. etrusca* and *D. liguriensis*, which we use in conjunction with transcriptomic data in order to search for genomic signals of the Meselson effect to confirm our hypothesis of ancient fissiparity in this group, presenting here our preliminary results.

## Evolutionary Biology: Session E3: talk

### **Subterranean sleeping beauty: Transcriptomics in long-term aestivation and arousal of Mediterranean earthworms**

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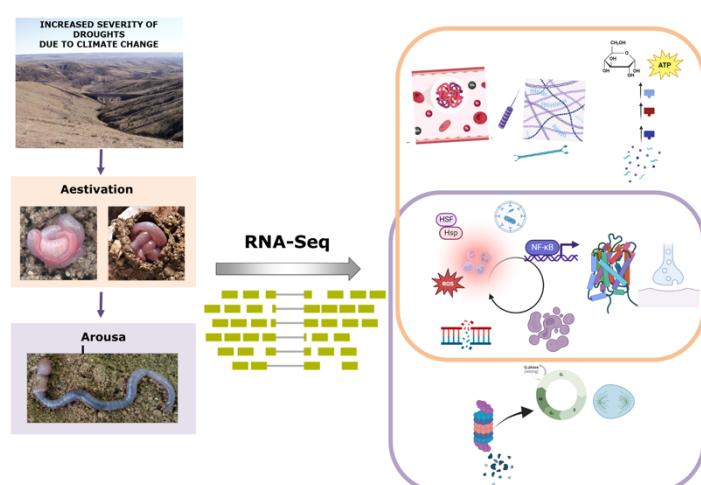
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Earthworms are crucial for maintenance of the biotic and abiotic soil properties, which is important for the biodiversity and productivity of terrestrial ecosystems, especially in the current scenario of climate change. Aestivation is a form of dormancy witnessed in organisms living in deserts or semiarid environments such as the ones found in the central part of the Iberian Peninsula. This study represents a continuation of our efforts to better understand aestivation and arousal of the endogeic Mediterranean species *Carpetania matritensis*.

The obtained results demonstrate for the first time ever that earthworms can aestivate for one year, and more importantly it describes the mechanisms for it. Aestivating earthworms reduce energy expenditure by fine-tuning their immune response focusing on the more imminent stressor: oxidative stress. Proteins against oxidative stress coordinate with cell fate determination and DNA repair mechanisms. Aestivation also causes a decrease of the stiffness of the extracellular matrix. These changes could be the key for ensured arousal of 1 year aestivating *C. matritensis*. Other biological processes also appear to be affected by aestivation; alternative metabolic pathways for energy sourcing could serve the increase of inhibitory neurotransmission. As for arousal, earthworms seemed to firstly reactivate their immune response to surrounding pathogens. Once they are safe and secure cell fate determination followed by which damaged cells were removed and tissues were repaired.

Figure 1. Graphical summary of the mechanisms of aestivation and arousal in *C. matritensis*.

The use of Spanish genetic resources was approved by the Ministry of Ecological Transition and Demographic Challenge (ESNC59).



## Evolutionary Biology: Session E3: talk

**The genomic basis of convergent adaptations to different subterranean environments**Tonzo, Vanina <sup>1\*</sup>, Muyle, Aline<sup>1</sup>, Isaia, Marco <sup>2</sup> & Nósil, Patrik <sup>1</sup><sup>1</sup> Center for Functional Ecology and Evolution, (CEFE-CNRS), Montpellier, France<sup>2</sup> Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy<sup>\*</sup>[vananatonzo@gmail.com](mailto:vananatonzo@gmail.com)

Convergent evolution, the independent development of similar traits in distinct lineages, is a common occurrence in nature and plays a critical role in shaping biodiversity patterns across all levels of biological organization. Organisms that have adapted to extreme environmental conditions provide an excellent framework for studying the repeatability of evolution. The sheet-weaver spiders of the genus *Troglohyphantes* (Araneae, Linyphiidae) represent the most species-rich spider lineage in European subterranean habitats. In the Italian Western Alps, *Troglohyphantes* species exhibit varying degrees of specialization and preferences for specific subterranean microhabitats. Interestingly, species inhabiting the same microhabitat are found in different cave systems, often isolated from each other by non-suitable habitats or geographic barriers. Because troglomorphic traits may be disadvantageous above ground, they are likely to have evolved independently (i.e., convergent traits) rather than being inherited from a common ancestor (i.e., conserved traits). In this study, we investigate whether environmental selective pressures determine patterns of genomic convergent evolution in these obligate subterranean organisms. Using a comparative genomic approach, we take advantage of the experimental conditions provided by multiple, independent subterranean microhabitats to characterize the genomic basis and tempo of subterranean adaptations to different microhabitats. Finally, we test alternative hypotheses about the origin of troglomorphic spiders and the parallelism in the underlying genetic basis of phenotypic adaptation to different subterranean microhabitats.

## Evolutionary Biology: Session E2: speed talk

### **Spatial Transcriptomics to investigate the origin of evolutionary novelties in marine invertebrates**

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A fundamental goal in evolutionary biology is understanding the origins of novel traits, structures with a form or function completely different to any previously known structure. The emergence of these novel traits—from new cell types to complex characters such as wings—often represent key evolutionary events, allowing lineages to occupy new niches and promoting species divergence. Recent advances in single-cell RNA-sequencing technologies, are transforming our understanding of the origins of novelty, dissecting the molecular mechanisms underlying the development of many traits. However, the specific position of cells within a tissue is often critical for its function and therefore, linking molecularly defined cell types with morphological, histological, and physiological data is fundamental to understand how novel traits arise. Spatially resolved transcriptomic methods can fill this gap by determining the specific location of molecularly defined cell types within tissues. Spatial Transcriptomics (ST) is a novel non-targeted technology that allows to visualize and quantify whole transcriptomes within histological sections at 100 µm resolution, enabling data-driven exploratory analyses without preselecting known targets of interest. We have optimized this technology to analyze spatial gene expression patterns at near-single cell resolution in several lineages of metazoans including sponges, annelids, and nemerteans, allowing us to investigate the origins of key evolutionary novelties such as gonads and venom systems.

## Evolutionary Biology: Session E1: talk

### Radiation across an extreme altitudinal gradient in a lineage of Austral Pacific arthropods

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Mountains are dynamic environments that contribute to the shape of diverse ecosystems by their steady change of biotic and abiotic factors, confining species to narrow conditions and leading species to habitat specialization. Adaptations of organisms to novel environments often involve key innovations, which can result in extreme adaptations that reduce the species' capabilities to deal with other environments. This may be the case for insects adapted to high altitudes, in which species frequently exhibit modifications such as melanism and reduced wings. However, the mechanisms by which a species successfully occupies a particular habitat remain unclear. For instance, the origin of alpine lineages is often attributed to dispersion from lowlands or other mountains. Nonetheless, in regions characterized by intense tectonic activity, the passive uplifting of pre-existent taxa is feasible.

A remarkable example of recent tectonically active area is the Australopacific region, which features some of the most diverse archipelagos. It incorporates New Guinea, an island of highly complex geological composition. The formation of its landmass has been the result of a massive orogeny since c. 8 Ma, resulting in an extensive tropical upper montane and alpine ecosystem, reaching elevations of approximately 5,000 meters. Simultaneously, Australia experienced substantial aridification, which likely led to a diverse groundwater fauna. Consequently, this region provides an excellent system for studying differential environmental pressures that may open new ecological opportunities to species.

In this study, we focus on *Limbodessus* diving beetles, which inhabit the entire Australopacific region from lowlands and underground aquifers in Australia up to elevations of 4,200 meters in New Guinea, encompassing multiple habitat

transitions. To gain insight into the evolutionary trajectories of *Limbodessus*, we used whole-genome sequencing data. Our analyses revealed three distinct trajectories: 1) passive uplifting and in situ speciation as a result of the New Guinea orogeny, 2) underground colonization triggered by the loss of habitat during Australia's extreme aridification, and 3) cross-oceanic colonization to the eastern Pacific archipelagos from the Sahul continent.

# Phylogeography & Population Genomics



## Population genomics: Session P2: talk

### **Characterization of inversions' frequencies across Iberia populations of *Littorina saxatilis* and their role in diversification**

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Population genomic tools have been widely applied to study fundamental evolutionary processes and unveil the factors that contribute to adaptation and speciation. While whole-genome sequencing is desirable for population genomics inference, the associated costs remain prohibitive, mainly when a high number of samples/populations needs to be analyzed. In this context, hybrid approaches based on whole-genome sequencing of a subset of samples complemented by SNP genotyping has shown to be cost-effective to study population structure, demographic history, the genetic basis of phenotypic traits, adaptation and speciation in non-model species.

Marine snails such as the rough periwinkle (*Littorina saxatilis*) show variable patterns associated with parallel divergence of ecotypes exposed to various environmental pressures across multiple intertidal shores. The populations of Iberia form an independent clade that offers unique opportunities to study the evolutionary processes influencing the species diversity at the southern range edge. About 20 chromosomal inversions have been identified using linkage disequilibrium patterns in northern populations. Recent results have shown that many of them are present in Iberia. However, we still need to extract the full potential from genomic information gathered across Iberian *L. saxatilis* populations to reconstruct the history of the genetic variation underlying ecotype divergence and speciation in this and other areas of its distribution.

In this study, we are using whole-genome sequencing data for *L. saxatilis* available for individuals from Iberia, to develop a SNP array in order to decipher diagnostic SNPs: i) linked to different arrangements within inversions; ii) between ecotypes; iii) between males and females; iv) phylogeographic-informative; and v) involved in adaptation, including to thermal stress/desiccation. We will show preliminary results of the diagnostic SNPs that will be applied to characterize hundreds of individuals from different populations across Iberia and understand the evolutionary role of inversions at the southern edge of the species range.

## Population genomics: Session P2: talk

### **Conservation genomics of the Emirati Leaf-toed Gecko (*Asaccus caudivolvulus*; Gekkota; Squamata), a Critically Endangered endemism of the United Arab Emirates**

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The Emirati Leaf-toed Gecko (*Asaccus caudivolvulus*), the only vertebrate endemic to the United Arab Emirates (UAE), is currently classified as Critically Endangered by the IUCN Red List and, as of early as 2022, it was only known to exist in two localities of the UAE coastline. Unfortunately, the first locality has been inaccessible since 2006 due to the development of a governmental infrastructure, while the second was destroyed almost in its entirety due to the construction of a hotel complex in 2015. Consequently, the status and prevalence of the only endemic vertebrate of the UAE remained unknown since 2015. However, in May 2022, following an expedition to the UAE, we confirmed the existence of *A. caudivolvulus* in the second location and discovered four additional populations of this UAE endemism. Unfortunately, most of these populations were facing heavy anthropic pressures. In an effort to start a conservation program for this species, we successfully assembled and annotated a chromosome-level reference genome for *A. caudivolvulus*, representing the first reference genome for the Gekkota family Phyllodactylidae. The genome assembly and polishing process involved the use of long-read (ONT) and short-read (Illumina) data, while scaffolding was implemented through Omni-C. Additionally, annotation was performed based on both available and de novo-sequenced full-length transcriptome data (PacBio Iso-Seq). Then, we examined the genomic variability, population structure and demographic history of the newly discovered populations of *A. caudivolvulus* and compared them to other Arabian and Iranian representatives from its genus. We achieved this by conducting whole genome resequencing of nine *A. caudivolvulus* samples and 15 samples from ten distinct *Asaccus* species.

Results showed some degree of differentiation among all *A. caudivolvulus*, despite some populations being as close as 2 km to each other. Demographic reconstructions revealed a steep and continuous decline in effective population sizes over the past million years, and the levels of heterozygosity

observed in all *A. caudivolvulus* specimens were lower than any other congeneric species. In conclusion, despite the discovery of four new populations of *A. caudivolvulus* in the wild, the species faces severe threats from intense anthropogenic development, habitat fragmentation between populations, and remarkably low levels of heterozygosity. These findings stress the urgent need for conservation measures to protect and ensure the survival of this species.

## Phylogeography & Population genomics: Session P1: talk

### **Chromosomal rearrangements, hybridization and more: the complex evolutionary history of *Dugesia* in the Corso-Sardinian archipelago**

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The Mediterranean region, renowned for its biodiversity, offers an excellent opportunity to study the mechanisms underlying evolutionary diversification due to its intricate geological past. Among the organisms that have experienced significant diversification in this area are planarians. Our study focuses on a specific lineage of planarians within the Mediterranean region, known as the Corsico-Sardinian clade. Within these islands, three endemic *Dugesia* species were identified in the last century: *D. leporii*, nowadays considered missing; *D. benazzii*, found across both islands, and with various karyological biotypes, ranging from diploid ( $2n = 16$ ) to hexaploid and aneuploid; and *D. hepta*, found in coexistence with *D. benazzii* in four hydrographic basins in Sardinia, featuring a distinct chromosome number ( $2n = 14$ ) unparalleled among *Dugesia* species in the Western Palearctic. A recent molecular study indicated that *D. benazzii* might be a species complex, with signals of potential hybridization.

In our present study, we have generated new genetic data at multiple levels to clarify the species status of *D. benazzii* and explore the evolutionary history of *Dugesia* in the archipelago. By utilizing a combination of nuclear and mitochondrial loci, we have confirmed that *D. benazzii* represents a species complex. Through additional sampling efforts on both islands, including published records and suitable niche modeling sites, we successfully collected representatives of all species, including the rediscovery of *D. leporii*. Employing ddRADseq data, we conducted phylogenetic and genetic structuring analyses, revealing a complex evolutionary history likely shaped by paleogeography, hybridization events, and chromosomal rearrangements. Additionally, we investigated the chromosomal rearrangement responsible for the distinctive karyotype of *D. hepta* by obtaining a chromosome-level genome and performing synteny analyses with genomes from two other Western Mediterranean *Dugesia*.

In conclusion, our study provides valuable insights into the evolutionary dynamics of *Dugesia* in the Corsico-Sardinian clade by integrating genetic, phylogenetic, and chromosomal analyses.

Population genomics: Session P2: speed talk

## Diversification patterns of the genus *Scaurus* (Coleoptera: Tenebrionidae) in the western Mediterranean Region

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The presence of closely related species whose distribution ranges widely overlap is a rare event in nature. The coexistence of sister lineages can play an important role in their evolution, either through the occurrence of hybridization processes and genetic admixture or through the reinforcement of differences and reproductive incompatibility. The genus *Scaurus* represents an ideal model for studying the evolutionary processes underlying co-distributed species. This group of darkling beetles, which is widely distributed in the Mediterranean basin, includes species with overlapping ranges co-distributed on both sides of the Strait of Gibraltar, such as *S. uncinus* and *S. gigas*. In this study, using mitochondrial (Cyt-b) and nuclear (ITS2) markers, we provided a preliminary phylogenetic hypothesis for the genus and reconstructed phylogeographic hypotheses for the aforementioned species to detect common patterns of genetic and geographic differentiation and infer the possible processes that could have influenced the formation of their lineages, as well as discuss the possibility of hybridization between them.

## Population genomics: Session P2: talk

**Lessons from pangenomes: Genetic variant richness enables global invasiveness to *Styela plicata***

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Biological invasions are a major threat to biodiversity. Therefore, monitoring genomic features of invasive species is crucial to understand their population structure and adaptive processes to plan future management actions. However, genomic resources of invasive species are scarce, hampering the study of the molecular bases for their success. Here, we present the reference genome of *Styela plicata*, one of the most widespread invasive species in seaports. Furthermore, by using whole genome sequencing data from 24 individuals from six populations distributed worldwide we build its pangenome. Here, we characterize polymorphic inversions in four chromosomes, accounting for ~15% of the genome size. These inversions are polymorphic through its distribution area and prevent detection of population structure. When these structural variants are removed from the analyses, we are able to identify main biogeographic barriers and accurately characterize population structure within regions. Moreover, we recover three major mitogenomic clades, one of them with a large insertion of 1000pb with several genes partially duplicated. Finally, we trace gene functions of each chromosomal inversion, driving genetic differentiation between biogeographic regions, and underlying mito-nuclear interactions. Our results suggest that structural and genetic richness might be responsible for the species success when invading new habitats, showing how genomic and structural variants contribute to species population structuring and adaptation processes.

## Phylogeography & Population genomics: Session P1: talk

### The population genomics of *Eunice* deep-water coral-associated polychaete worms in the NE Atlantic

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Little is known regarding the taxonomic diversity and population connectivity of coral-associated invertebrate fauna in the deep sea. Here we present the population genomics of predatory polychaetes within the *Eunice norvegica* species complex inhabiting the skeletons of living cold-water corals in the NE Atlantic at depths ranging from 200-1,200 m. Analyses of mitochondrial 16S sequences of 181 specimens collected from seamounts to the northwest of the UK and the Cantabrian Sea indicated the presence of four different species. Two of these species included *Eunice norvegica*, found at all sample sites, and *Eunice* sp., only found at the Anton Doroñ seamount at depths exceeding 1,000 m. Traditional markers and RADsequencing-derived SNPs confirmed that *Eunice norvegica* and *Eunice* sp. are sister species, possibly reflecting cryptic adaptation to depth regimes and/or host specificity. Within *E. norvegica* population genetics/genomics analyses on both the 16S and the SNP datasets agreed in detecting a single panmictic population despite samples being separated 100s of km and also being collected within a depth range of ca. 1,000 m. These results contrast with those recently published on a common sponge collected from similar areas but reflecting a clear genetic structure between the Cantabrian and the rest of areas.

## Population genomics: Session P2: speed talk

### **Allopatric and ecological diversification in *Cyaniris semiargus* (Lepidoptera, Lycaenidae)**

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*Cyaniris semiargus*, the Mazarine blue, is a relatively common Lycaenidae in the western Palaearctic, typically inhabiting grasslands and feeding on various Fabaceae species. It is widely distributed across most of its range but has somewhat isolated populations at mountain ranges at the southern part of its range.

Previous studies on mtDNA revealed three lineages in southern isolates, one of which also individually represents one of two evolutionary significant units and corresponds to a population from Doñana, southwestern Spain. This population represents an ecological contradiction: it occupies sandy areas at or slightly above sea level and the larvae feed exclusively on *Armeria velutina* (Plumbaginaceae). Another population with the same apparent ecological needs was later found in Aljezur, southwestern Portugal.

Our aim was to explore these lineages and patterns of differentiation with ddRADseq data. Results suggest the existence of two different lineages in the southern isolated populations and very low levels of admixture between these and the widely distributed main lineage. Results also indicate mild to strong divergence of each southern isolate. Further analyses on gene flow, species delimitation, ecology and morphology need to be done, but we have solid indication that these populations present unique diversity important to consider in terms of conservation.

## Population genomics: Session P2: talk

### Adaptation and divergence of *Marthasterias glacialis* based on single nucleotide polymorphisms

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Previous molecular studies revealed the presence of two distinct mitochondrial lineages in the voracious sea star *Marthasterias glacialis* along the Atlantic-Mediterranean region (Pérez-Portela et al., 2010, 2017). Nevertheless, the scarcity of nuclear data did not allow determining whether these lineages actually corresponded to two different evolutionary units and/or species. Furthermore, there is still a lack of information regarding gene flow and local adaptation patterns in this widely distributed species ranging from the cold waters of Norway to the warm Eastern Mediterranean. To address these questions, herein we applied a double digested Restriction-site Associated DNA sequencing (ddRAD-Seq) protocol for 258 individuals collected from 19 locations, spanning almost the entire distribution range of the species. From the ddRAD-Seq data, we identified 18,589 Single Nucleotide Polymorphisms (SNPs) that were suitable for population genomics and local adaptation analyses. Our results revealed the absence of nuclear divergence between the mitochondrial lineages. However, significant differences were observed among populations and the three main geographical areas analyzed: the continental Atlantic, the Azores, and the Western Mediterranean from the neutral SNPs. These differences were attributed to the isolation of populations based on geographical distances. Additionally, evidence of local adaptation to temperature and salinity were also detected. We identified 862 candidate SNPs under selection to salinity and 927 to temperature. Therefore, our results suggest that both neutral geographical processes promoting gene flow interruptions and local adaptation to local environmental conditions have shaped the genomic structure of *M. glacialis*.

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Population genomics: Session P2: speed talk

## **Following the arid tribe Adesmiini footsteps (Coleoptera: Tenebrionidae) across deserts worldwide**

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Taking into consideration the progressive desertification of many of the World's ecosystems, identify biotic factors (interaction between species, demography, ecology, etc.) and abiotic factors (palaeogeography, paleoclimatology etc.) that have determined current distribution ranges of desert species and evolutionary success is a challenge. The tribe Adesmiini Lacordaire, 1859 (Tenebrionidae: Pimeliinae) lives in one of the warmest and driest environments on Earth throughout African and Western Palearctic areas. With both diurnal and nocturnal species, this tribe constitutes a great example maximizing thermal tolerances with many desert-specified adaptations. A first phylogenomic hypothesis of the tribe based on 529 genetic loci of 43 species, supports the monophyly of the tribe and at the same time exposes a complex taxonomic scenario. Ancestral state reconstruction analysis for activity patterns indicated that the most recent common ancestor of Adesmiini was likely diurnal. Here, we will establish an evolutionary time frame of diversification, conduct biogeographic reconstructions to estimate ancestral areas of origin, vicariant and/or dispersal events, explaining its current disjunct distribution, and colonization patterns on desert biomes.

## Population genomics: Session P2: speed talk

### A genomic analysis of *Diadema africanum* populations: an engineering species in the West African region

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The sea urchin *Diadema africanum* is a significant macro-herbivore found in the rocky reef systems of the West African region and the Macaronesian archipelagos. This species has experienced a notable population increase over the past few decades, which in many cases has drove to an impoverishment of coastal rocky substrates, resulting in the formation of barren grounds due to its high grazing activity. However, recent mass mortality events during 2010 and 2018 related to the presence of pathogens, have promoted strong population declines of this species in some islands, with a potential impact on their genomic diversity. To date, no detailed phylogeographic or population genetic studies have been performed to test the effect of population decimation on genetic diversity and population structure, as well as the role of connectivity among distant areas in promoting population recovery. In this study, we analyse population structure and diversity levels, as well as local adaptation patterns in *Diadema africanum* across part of the species distribution range. This included populations that passed through mass mortality events and others that were not affected by pathogens. To address our objectives, we used 9,109 Single Nucleotide Polymorphisms (SNPs) isolated from a ddRAD-seq protocol and a fragment of a mitochondrial gene. Our research provided compelling evidence of low genetic diversity in this species and, in general, the absence of population differentiation across the studied area when all markers were used together. The high dispersal potential of the larvae in this species allows high connectivity among islands, homogenising population structure and favouring population recovery after mass mortality events. The observed genetic features can be attributed to the species' divergence from a recent ancestor and a small genomic pool, followed by a strong founder effect and the current process of demographic expansion. However, sub-structure within Canary Islands localities was observed from 405 SNPs identified as candidate loci under selection, potentially associated with local adaptation to temperature.

Population genomics: Session P2: speed talk

**Assessing land cover influence on dispersal and connectivity between Iberian populations of *Chirocephalus diaphanus* Prévost, 1803  
(Anostraca: Branchiopoda)**

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Genetic diversity enrichment achieved by genetic connectivity between populations is essential to ensure the continuity of species. Promoting of connectivity is possible through the management of populations through their environment. This involves the knowledge about the ecological elements that positively and negatively influence the movement of the organisms of interest. Landscape genetics studies could make this information available through analyses of correlation between genetic differentiation and the effective distances of chosen environmental variables.

Anostracans (Branchiopoda) is an order of crustaceans that live in temporary and continental aquatic ecosystems. When the pool is near to drying up, adults deposit eggs in the sediments which are able to resist years of drought. This condition is hence accompanied by passive dispersal performed by biotic and abiotic agents such as wind, mammals, amphibians, insects or birds. Therefore, conducting a landscape genetics study in anostracans involves choosing environmental variables that influence on the possible active dispersal agents.

In this study, we chose land cover as a potential variable that may be interacting with Iberian populations of the species *Chirocephalus diaphanus*.



We sampled populations surrounded by two different land covers: agricultural and natural lands. Our purpose is to test if any of these two types of landscape has a positive or negative impact on genetic connectivity between populations of the species throughout the studied area.

## Phylogeography & Population genomics: Session P1: talk

### **Phylogeography and conservation of *Melitaea diamina* (Lepidoptera, Nymphalidae): southern relict populations trapped by climate change**

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*Melitaea diamina*, commonly known as the false heath fritillary, is a butterfly species found mainly in temperate areas of Europe and Asia. The species has a patchy distribution, and its populations, especially the southernmost ones, are often scattered on mountain areas and isolated from one another. This suggests that they represent relicts of the species' more extensive range during the last cold phase. Previous studies using mitochondrial DNA data indicated the presence of several lineages in Europe, with the notable differentiation of certain geographically isolated Iberian populations. In this study, we investigated European populations of *M. diamina* in order to unravel the origin of the multiple Iberian lineages and find possible explanations. To achieve the desired level of detail, we generated a ddRADseq dataset, which we subsequently used to perform various analyses, ranging from phylogenomics to model and non-model based population genomics, and demographic analyses. The results follow a pattern similar to what has been found in other Lepidoptera species, showing limited genetic diversity across the majority of the species' range, but with a gradual increase towards the Iberian Peninsula. The analyses indicated the presence of at least three different lineages within the peninsula, with minimal genetic admixture between them. Some of these lineages correspond to genetically unique and geographically isolated populations, that are restricted high-altitude areas and therefore would be threatened by further temperature raise brought by climate change. These findings highlight the importance of preserving these newly discovered evolutionary significant units and their corresponding habitats.



# Posters

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## New insights on the diversity of bone-eating worms (Osedax, Siboglinidae, Annelida) around the Iberian Peninsula

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Annelid worms of the genus Osedax (Annelida, Family Siboglinidae), commonly known as “bone eating worms”, are bone specialists found in almost all the seas and oceans in the planet. These fascinating creatures lack mouth and gut and they rely on an obligate symbiosis with bacteria hosted in their roots to feed on the organic matter retained in the bones. Since they were first described in 2004, a total of 26 different species have been named, in addition to approximately five new potential species that remain unnamed. In the NE Atlantic only one shallow-water species has been reported, *Osedax mucofloris*, a species that has also been found in the coast of Portugal at ca. 1000 m depth.

In the present study we used cow and pig bones experimentally deployed at different depths (100, 500 and 1000 m) around the Iberian Peninsula in order to uncover the diversity of Osedax worms of this region. Experimental settings were deployed for 3–9 months in three different regions from the coast of Spain: the Cantabrian Sea, the Gulf of Cádiz and the coast of Mazarrón (Murcia). Here, we describe the occurrence of two new species to science of the genus Osedax using a combined morphological and phylogenetic approach. One of the species was found in the Cantabrian Sea at ca. 500 m (*Osedax ‘cantabrian’*) and the other two were found in Mazarrón at 100 (*Osedax ‘mediterranea’*, already reported from the Catalan coast) and 1000 m (*Osedax ‘mazarrón’*), respectively. *Osedax ‘mediterranea’* presented pinnules in the palps, while the other two did not. Interestingly, both *Osedax ‘cantabrian’* and *Osedax ‘mazarrón’* presented patches in the anterior part of the trunk with slightly different coloration. Phylogenetic analyses using five genetic markers (COI, 16S, 18S, H3 and 28S) confirmed that the two species are indeed new to science and confirmed the second report of *Osedax ‘mediterranea’* in the Mediterranean Sea. It is worth noting, that none of these three species reported here are phylogenetically close to *O. mucofloris*, the closest Osedax geographically. These three species studied here are currently being formally described and will significantly expand the knowledge of this group of marine invertebrates in Atlanto-Mediterranean deep-water habitats.

## Which are the most useful nuclear and mitochondrial genes for molecular systematics in marine flatworms?

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Increases in the diverse use of molecular methodologies in ecology and systematics have driven the necessity for a more comprehensive understanding of the benefits and limitations of different genetic markers employed. Specifically, given the multiple existing approaches for the study of the taxonomy and systematics of Polyclad flatworms and the increasing use of molecular tools in the research of their phylogeny, the objective is to evaluate the usefulness of a range of nuclear ribosomal (18S rDNA, 28S rDNA) and mitochondrial ribosomal (16S rDNA, Cytb, Cox1) genetic markers.

We estimated the rates of substitutions of the studied markers, finding Cytb the most variable and 18S rDNA the least variable among them. We identified the transition to transversion (Ti/Tv) ratio of the different genes, concluding that overall, the number of transversions is higher than that of transitions in this taxon. Mutation rates and Ti/Tv ratios of the different genetic markers were assessed in polyclad flatworms for the first time.

Lastly, the results show that the third codon position of the studied protein-codifying genes was highly variable, observing this position is saturated in the Cox1 marker but not for Cytb. Future studies should focus on the use of mitochondrial genes (Cytb and Cox1) when analysing phylogenetically closely related species and the 28S rDNA nuclear marker for resolving higher taxonomical clades.

## Disentangling the genomic basis of terrestrialization across animals through a transcriptomics spyglass

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Terrestrialization - the colonization of the land from aquatic animals, plants and fungi - is one of the most remarkable events in the history of life on Earth. From a relatively stable marine environment, animals changed the way they breathe, excrete, reproduce or smell in order to adapt to such an extreme change of environment. Out of the 36 phyla in the Animal Tree of Life, 9 comprise terrestrial forms (including arthropods, annelids, mollusks, nemerteans, nematodes, tardigrades, platyhelminthes, craniates and onychophorans). Despite the evolutionary relevance of this transition, the genomic basis of biological adaptation to life on land is largely unexplored. The unveiling of the genes of terrestrial animals - and their aquatic relatives – that facilitated the adaptation to such an extreme environment will shed light onto this complex evolutionary phenomenon. To tackle this, we exposed terrestrial animal species and their aquatic close relatives per phylum to a series of abiotic stressors mimicking those that animals had to face to adapt to life on land (including exposure to UV light, responses to chemosensory cues, and changes in oxygen concentration, salinity and humidity), totalling 12 experiments in 23 species from the 9 animal phyla that colonized land, and that resulted in ca. 1,000 transcriptomes sequenced through a combination of Illumina and PacBio Iso-Seq. Through the lens of comparative transcriptomics and comparative phylogenetic methods, we are exploring which genes, gene families and gene networks are potentially involved in a response against these stressors, and how they may have facilitated adaptation to life on land. The results of this project will shed light on how animals reshaped their genomes to cope with the dramatic physiological and morphological changes associated with life on land.

## Species complexes as windows into diversification drivers of speciose lineages: the *Dysdera erythrina* red devil spiders (Araneae: Dysderidae)

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The red devil spiders, family Dysderidae (Araneae, Dysderoidea), are well-suited for exploring the drivers of asymmetries in species richness across lineages. Two of its 25 genera account for 80% of the family diversity, and groups of very closely related species are common within the most diverse genera. In particular, the type species *Dysdera erythrina* (Walckenaer, 1802) is part of a complex of 20 similar, recently diverged (i.e. Pleistocene) species mostly restricted to the northeastern Iberian Peninsula. Although species slightly differ in genital and cheliceral morphology, they show remarkable differences in karyotypes, ranging from 9 to 20 in chromosome number. Because of its recent diversification, the *D. erythrina* complex may showcase the microevolutionary processes responsible for the high diversification rates of the genus (~300 species). Here, we used double-digest restriction-site associated DNA sequencing (ddRADSeq), and the information provided by the chromosome quality genome of one of the complex species, to generate SNP data from 216 samples from all the species distributed across the northeastern Iberian Peninsula. Based on these data, we unravel patterns of genetic diversity and population structure within the complex, examine species boundaries and relationships and test the role of geography and ecology in the diversification of the group.

## Large differences in genetic parameters between populations of an endangered mammal: the Iberian desman

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The Iberian desman (*Galemys pyrenaicus*) is a semiaquatic mammal endemic to the north of the Iberian Peninsula whose populations have experienced a sharp decline in recent years. Effective conservation of this threatened species requires a deep understanding of its demographic, ecological and genetic aspects. However, from a genetic point of view, it is still difficult to decide which parameters are the most suitable to describe the populations and predict their viability. In this work, we compare ddRAD sequences of more than 200 specimens from several populations of the Iberian desman: three in strong regression (Central System, Iberian Range and western limit of the Pyrenees) and one more stable (Sanabria region of the West of the Iberian Peninsula). We analyze different genetic parameters, including population genetic diversity, individual heterozygosity, inbreeding coefficient and genetic connectivity during the last generations, and examine their variability within and among populations. Although it has been pointed out that there is a correlation between some of these parameters, this is only partly true within each population, but not for the species as a whole. Therefore, it is necessary to analyze all these parameters as well as others that can be obtained from whole genome analysis to properly describe the genetic status of each population and detect the most vulnerable ones. Although it is still challenging to predict the fate of each population, our study highlights the importance of analyzing multiple genetic factors to assess the conservation needs of endangered species.

## Species boundaries to the limit: integrating species delimitation methods is critical to avoid taxonomic inflation in the case of the Hajar Banded Ground Gecko (*Trachydactylus hajarensis*)

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The availability of Next Generation Sequencing techniques and the implementation of Multispecies Coalescent-based (MSC) species delimitation methods (SDM) especially for non-model organisms has increased the number of taxonomic studies unveiling, and describing new cryptic species. However, speciation between early divergent lineages is often defined without evaluating population structure or gene flow, which can lead to false claims of species status and, subsequently, taxonomic inflation.

For this study, we have focused on the Arabian gecko *Trachydactylus hajarensis*. We generated mitochondrial data (12S rDNA) and genome-wide SNP data (ddRADseq) for 52 specimens to determine phylogenomic relationships, population structure and genetic diversity within this species. Then we applied a set of different SDMs to evaluate several competing species hypotheses, through the MSC.

Results indicate that *T. hajarensis* is comprised of 3 well delimited populations, two in the Hajar Mountains and one on Masirah Island. Even though we did not find clear evidences of current gene-flow, mitonuclear discordances were recovered between mainland populations. Surprisingly, discordances in species tree topology were found when different downsampled datasets were used, especially when linking population sizes. Additionally, different SDMs yielded different results, supporting from four species within the group, to *T. hajarensis* being a single species.

We believe our findings enhance the importance of both sample and prior choice, as well as of the integration of various species delimitation methods in order to provide thorough and meticulous taxonomic studies.

## Assessing biodiversity and anthropogenic impacts in the Mar Menor using environmental DNA metabarcoding through sponge tissue collection

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Monitoring is a fundamental tool for combating biodiversity loss, gathering valuable information that can be used to develop conservation policies, evaluating management outcomes, and guiding science-based decision-making. Then, knowledge about the composition of organisms in a particular ecosystem is crucial for developing more reliable and tailored conservation strategies for ecosystems. The Mar Menor is a Mediterranean saltwater coastal lagoon, located in southeastern Spain. It is the largest Spanish lagoon and possesses unique environmental values, but it has been experiencing episodes of advanced eutrophication due to an excess of nutrients, primarily originating from intensive agriculture and other human activities. These have resulted in several incidents of mass mortality of marine fauna, causing a significant loss of biodiversity in this ecosystem, and therefore, biodiversity monitoring in this location is particularly important to help evaluate the situation and address it properly. Environmental DNA (eDNA) metabarcoding, obtained from the filtering of water samples, has been increasingly used to monitor the community assemblages of a wide variety of organisms in marine ecosystems. Alternatively, instead of artificial filters, sponge tissue has been shown to naturally trap and concentrate the particles from which environmental DNA is isolated, thus acting as a natural filter. Our main objective was to assess the biodiversity composition and structure patterns in the benthic ecosystems of the Mar Menor through metabarcoding of eDNA using the cytochrome oxidase I gene (COI), collected from sponge tissue. We evaluated the biodiversity in two different areas with different levels of anthropogenic activities and during 2 different seasons with different levels of contaminant disposal. We discuss our results in the context of the biodiversity estimations of a well-known area and how the recent increments of anthropogenic pressures are changing the composition and structure of metazoan biodiversity in the area. The results of this study will contribute to a better understanding of the organism community in the Mar Menor, as well as to identify which communities in this ecosystem are more sensitive to contaminants and anthropogenic effects.

## The atlas of mitochondrial genetic diversity for Western Palearctic butterflies

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Butterflies are a fascinating group of insects and their study has become increasingly important in the context of global biodiversity conservation efforts. This group is the most extensively studied with mtDNA sequencing, which has proven particularly useful for species identification (DNA barcoding), detection of cryptic species, reconstruction of phylogeographic phenomena, and evaluation of matriline dispersal. The atlas of mitochondrial genetic diversity provides a full set of resources for the study of Western Palearctic butterflies: (1) an updated checklist comprising 555 species; (2) a curated dataset of ~33,000 COI sequences for 534 species; (3) seven indexes of intraspecific genetic variation (IGV); and (4) an atlas with species-level maps illustrating their phylogeographic patterns. Version 2.0 of the atlas of mitochondrial variation with recently updated taxonomy and sequence data is available on GitHub ([www.github.com/leondap/iodatabase](http://www.github.com/leondap/iodatabase)), together with the IOdatabase project as an R package.

We hope that this resource will enhance the research on butterfly diversity and evolution in the Western Palearctic.

## Monitoring cetacean biodiversity using environmental DNA

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Studying the pool of DNA concentrated from an environmental sample has become a powerful approach for studying its biodiversity. Cetaceans are highly specialised, apex predators and key components of ocean ecosystems. This study aims to use non-invasive methodologies coupled with high-throughput metabarcoding sequencing for accurate cetacean monitoring in the Cetacean Migratory Corridor, a recently declared Marine Protected Area in the Western Mediterranean.

We collected 62 seawater samples from around Columbretes Islands ( $n=18$ ) and the Ibiza Channel ( $n=44$ ) covering all seasons during 2021 and 2022. Samples obtained from Columbretes were associated with specific cetacean sightings, while samples from the Ibiza Channel were systematically collected from previously established sampling points during each season. Water was collected using a manual pump in Columbretes and Niskin bottles in the Ibiza Channel at a maximum depth of 5m, stored in metalized bags and kept cold until filtration at the laboratory. Water was filtered using Sterivex filter capsules and using eDNA metabarcoding, we targeted amplicons of 235-290 bp of the 16S RNA gene using vertebrate-specific primers. Filtration blanks were included in order to check for cross-contamination. A standard bioinformatic pipeline, including DADA2, was used to curate the amplicon reads and infer amplicon sequence variants (ASV), which were identified taxonomically with the NCBI Nucleotide database.

A total of 3,884,606 raw reads was obtained, from which after curation and trimming for the targeted amplicon size, only 473,477 reads (12.18%) were kept for taxonomic assignment. There were 358 ASV, of which 148 were assigned to Metazoa and of these, 135 were assigned to Chordata. More than a half of the obtained ASV remained taxonomically unassigned (210 ASV, 58.7%), which suggests that a thorough revision of the sequences obtained and the reference database is urgently needed. From the target taxa 57 ASV were identified as Actinopterygii, 6 as Chondrichthyes, 1 as Chelonidae, 1 as Phocidae and 11 as Cetacea. Known cetacean species occurring in the region were identified using eDNA from water samples, such as *Tursiops truncatus*, *Delphinus delphis*, *Grampus griseus*, *Stenella coeruleoalba* and

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*Balaenoptera physalus*. Taxonomic identification of 3 ASV remained as Delphinidae and one as *Orcinus orca*, which even though is a cetacean species reported in the Mediterranean, this could represent a misidentification or a incongruent match in the nucleotide database. Our study shows that genomic-based technologies offer an unprecedented opportunity to advance in the development of cetacean monitoring systems. However, these results conform a pilot study where refinement of collection and filtration techniques and bioinformatic processing of reads shall be improved. This study is supported by projects CETABIOENA (CISEJI/2022/5) and IOS4DOM (CIDEVENT/2021/058) from the Talented Research Support programme PlanGenT from Generalitat Valenciana.

## Tales from the genome: insights into the origin and evolution of giant genomes in whisk ferns (genus *Tmesipteris*, *Psilotales*)

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Giant genomes are rare in the plant kingdom and their study has mainly focused on angiosperms and gymnosperms, while ferns have received less attention. *Tmesipteris* is a small genus comprising approximately 15 species of mainly epiphytic ferns found in Oceania and several Pacific Islands. So far, the genome size of two *Tmesipteris* species have been measured using flow cytometry, confirming the presence of giant genomes in the genus [i.e. *T. tannensis* ( $1C = 73.19$  Gbp) and *T. obliqua* ( $1C = 147.29$  Gbp)]. In order to better understand the origin and evolution of the genus we have built the first complete phylogeny of *Tmesipteris* using a self-designed sequencing kit amplifying 288 single copy genes. In addition, full chloroplast reconstructions have been used to investigate patterns of nuclear and chloroplast evolution, enabling delimitation of species boundaries and the diversification of the genus in the Pacific. New genome size data have been generated in two species, complemented with ploidy levels inferred based on changes in stomatal guard cell size and available chromosome counts. Additionally, the composition of the repetitive DNA fraction of the genome for each species has been identified and quantified through low-coverage genome skimming sequence data analysed with the RepeatExplorer2 pipeline. Our results indicate that species share a similar genomic composition, with high repeat diversity compared to taxa with small ( $1C < 10$  Gbp) genomes, and characterised repetitive elements have relatively high heterogeneity scores, indicating ancient diverging evolutionary trajectories. These findings suggest at least one whole genome multiplication event, accumulation of repetitive

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elements, and recent activation of those repeats have played a role in shaping these giant genomes. Future research will be focused in and in-depth investigation into the evolutionary history of the genus and further mapping of ecological and geological variables to be able to fully understand all factors contributing to the rise of these giant genomes.

## Jumping through hoops: Structural rearrangements and accelerated mutation rates on mitogenomes reshape the family Dendrodorididae (Mollusca, Heterobranchia, Nudibranchia)

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The family Dendrodorididae (which includes only two valid genera) has been a challenging group for integrative taxonomists. Its members lack hard structures for morphological comparisons, and their mitochondrial and nuclear markers provide contradictory phylogenetic signals. These conflicting results were hypothesized to be the result of exogenic contamination or the presence of nuclear pseudogenes, which disrupted phylogenetic relationships. However, these proposals have never been empirically tested. In the present study, by using genomic data of seven Dendrodorididae species, we test these hypotheses while tackling the evolutionary processes underlying this family. Two of the mitogenomes display a unique structural rearrangement for nudibranchs, involving a translocation of three genes and surrounding tRNA. When coding sequences are aligned, blocky regions of non-synonymous mutations are found in the distinct mitogenomes, as well as multiple insertions and deletions. Both signals provide evidence of a fast evolutionary process in Dendodordidae mitogenomes. Protein structure modelling corroborates the functionality of all protein sequences, as well as their protein complexes, validating the orthology of genetically distant sequences. Phylogenies using whole mitogenomic data display a specific clade for the rearranged mitogenomes, although they fail to clarify the family relationships. With the incorporation of nuclear data, the taxonomy of the Dendrodorididae is further resolved by recovering additional clades, although further work needs to be carried out to completely resolve the family systematics. The present work provides novel evidence on molecular jumps as speciation triggers and highlights the relevance to use genomic data to unveil uncommon evolutionary processes before inferring phylogenetic studies.

## Integrative taxonomy of a species of the genus *Macellicephalia* (Phylum Annelida, Family Polynoidae) associated with a carnivorous sponge from the Arctic

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The polynoid family consists of polychaete annelids that are commonly involved in numerous symbiotic relationships with a large variety of marine invertebrates. In this study, we describe a curious symbiotic relationship between the carnivorous sponge species *Cladorhiza oxeata* and a polynoid of the genus *Macellicephalia*. This is the third time a polynoid has been described as a symbiont of a carnivorous sponge and the first time a symbiosis between a sponge of the genus *Cladorhiza* and a polynoid has been described. The detailed description of the morphological characteristics of the polynoid, using optical and scanning electron microscopy, as well as its phylogenetic placement using three genetic markers (16S, 18S, and COI), suggests that the worm is a new species of the genus *Macellicephalia*. However, the symbiotic relationship between the polynoid and the carnivorous sponge does not appear to be obligated, as described, for example, in the case of the also polynoid *Neopolynoe chondrocladiae* with sponges of the genus *Chondrocladia*. Nevertheless, this can only be confirmed when a greater number of samples are studied. The haplotype network obtained from the COI of the four studied polynoids indicates at least three colonization events of the sponge had happened.

## Towards the identification of the molecular toolkit involved in scale worm bioluminescence (Polynoidae, Annelida)

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Bioluminescence, or the ability to produce light by a living organism, is present in numerous taxa inhabiting different ecosystems, although it is more frequent among marine animals. Polynoids or scale worms, are a group of marine annelids characterized by having dorsal scales, known as elytra, capable of emitting bioluminescent light. Here, we used RNA-seq data to characterize genes potentially involved in light production in the polynoid species *Harmothoe imbricata* (Linnaeus, 1767) and *Harmothoe areolata* (Grube, 1860). We also compared the transcriptomes of the selected species with other bioluminescent and non-bioluminescent polynoids, to identify additional shared orthologous genes potentially involved in light production. Our results showed a total of 16 genes, 15 clusters and 12 enriched GO terms potentially implicated in bioluminescence, including genes related with oxidative stress, cytoskeleton, nervous system, stress response, wounding response, eye constituents and metabolic pathways. In addition, we investigated the disposition of the photocytes on the elytra using confocal microscopy and histological analyses, confirming the light-producing cells of *H. imbricata* are distributed around the elytrophore. Our work sheds light on a unique and poorly known bioluminescent system providing valuable molecular resources for future studies of the origin and evolution of bioluminescence in annelids.

## Spatio-temporal reconstruction of a Neotropical radiation: the case of *Hypericum*

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The extraordinary species richness of the Neotropics has raised many questions about the evolutionary and/or ecological factors influencing the origin of this biodiversity (VonHumboldt 1806; Stebbins 1974; Gentry 1982). Continental isolation and tropical climate stability have traditionally been proposed as factors promoting diversification in the Neotropics (Simpson, 1980), however, in the last decade, it has been suggested that environmental instability (geological and environmental disturbances) has been responsible for Neotropical diversity (Gentry 1982; Hoorn et al. 2010; Rull 2011; Antonelli et al. 2018a). The plant genus *Hypericum* L. (Hypericaceae) is one of the genera that experienced rapid radiations in the Neotropics (Madriñan et al., 2013). In this work, we aim to understand the processes that mediated species diversification in the Neotropics using *Hypericum* as a model, by inferring an almost complete phylogenetic hypothesis for the radiation of *Hypericum* in the Neotropics, and representative lineages for the main clades of the genus in the New World (clade to which the Neotropical lineages belong). To answer this question, we will use 'Hybrid enrichment with genome skimming (HybSeq)' techniques and the Clusioid baits kit, designed to capture 626 low-copy nuclear genes. In addition, we will use an integrative statistical approach to infer the evolutionary and biogeographical factors that explain the diversity of the genus in the region, and to infer the role of climate change and Andean orogenesis in the radiation.

## Solving the evolutionary position of the Franco-Iberian endemisms *Prosellodrilus* and *Cataladrilus* in the phylogenetic Lumbricidae tree and clarifying the evolutionary relationships between their species

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The Iberian Peninsula and south-western France seem to be a centre of diversification of a wide variety of endemic earthworm species and genera within the Lumbricidae family. However, most of those have been studied based solely on morphological characters, and evolutionary relationships between the genera and between the species belonging to each genera are still uncertain. In the present study, phylogenetic analyses were carried out with the molecular markers COI, 16S, 28S and ND1 in order to clarify the phylogenetic position of the Iberian and South France endemisms *Prosellodrilus* and *Cataladrilus* in the lumbricid tree. Calibrated phylogenetic trees were obtained in order to understand their historical biogeography and shed light into the early evolutionary history of Lumbricidae, and their morphological diversity was related to their phylogeny.

Phylogenetic trees recovered all species of the genus *Prosellodrilus* (including the type species *P. idealis*) in a monophyletic clade, as a sister clade to a monophyletic *Cataladrilus*: they are thus two well supported genera which appear to be closely related to other early-branching Lumbricidae like the Iberian endemism *Castellodrilus*.

Regarding the evolutionary relationships within *Prosellodrilus*, some subspecies such as *Prosellodrilus pyrenaicus aragonicus* or *Prosellodrilus psammophilus magnus* were found to be unrelated to representatives of their parent species (*Prosellodrilus pyrenaicus* and *Prosellodrilus psammophilus*), hence they are proposed to be elevated to species status. Based on phylogenetic relationships and genetic distances further taxonomic revisions (such as the synonymy of *Prosellodrilus trigoi* and *Prosellodrilus pyrenaicus aragonicus* were suggested). *Prosellodrilus festae* and *Prosellodrilus amplisetosus* were recovered as the earliest branching species, placing the origin of the genus in Sardinia-Southern France, while most of the Iberian species were recovered within a single derived clade. This suggest that a single colonization event of the Iberian Peninsula resulted in a large taxonomic and functional diversification, with large endogeic species occupying drier habitats.

These results serve as a base to understand the evolutionary and ecological significance of this diverse but usually neglected earthworm group.

## De novo genome hybrid assembly and annotation of the endangered and euryhaline fish *Aphanius iberus* (Valenciennes, 1846) endemic from the Mediterranean wetlands of the Iberian Peninsula

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The sequencing of non-model species has increased dramatically in recent years due to the revolution brought by new sequencing technologies. In this study, we used a hybrid assembly approach that combined PacBio long-read sequencing with Illumina short-read data to generate the reference genome of *Aphanius iberus*, an endemic endangered fish from the Iberian Peninsula. It is also the first Whole Genome Sequencing (WGS) Euro-Asian species within the globally distributed order Cyprinodontiformes.

This research is particularly interesting as *Aphanius iberus* has developed unusual adaptations to thrive in dynamic and ever-changing inland aquatic habitats along the eastern coast of Spain, ranging from groundwater springs to salt marshes. The genome assembly was annotated using AUGUSTUS, and two different approaches were employed for functional annotation. The size of the genome aligns with that of the most closely related species, and its quality and completeness, assessed with various methods, exceeded the suggested minimum thresholds, thus confirming the robustness of our assembly.

The integration of genomic data with predicted genes presents future research opportunities in various fields, such as physiology, reproduction, disease, and opens up new avenues for future studies in comparative genomic studies. Overall, this study contributes to the growing database of whole-genome sequencing, provides valuable information that can enhance the knowledge within the Cyprinodontiformes order, and aids in improving the conservation status of threatened species by facilitating a better understanding of their behavior in nature and optimizing resource allocation towards their preservation.

## Comparative landscape genomics in cosmopolitan and Mediterranean endemic earthworms

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Some earthworm species have shown their capacity to acclimate or adapt to diverse climate conditions. Within a context of climate change, those species that present variable loci in genes involved in functional responses to changing climate variables may have more options to rapidly adapt to locally changing environments. Understanding the effects and distribution of potentially adaptive alleles putatively associated with a better acclimation to a varying environment could aid to predict the responses of species to the future conditions and to mitigate biodiversity loss. *Aporrectodea trapezoides* Dugès, 1828 is one of the most widely distributed cosmopolitan species in temperate habitats: their geographical distribution (which includes a range of different climatic conditions) make it an ideal candidate to explore the relationship between their genomic variability and climate (landscape genomics). Earthworms belonging to the genus *Carpetania* Marchán et al. 2017, while way more geographically restricted (they are endemic to Central Iberian Peninsula) have demonstrated a remarkable adaptation to the harsh, strongly seasonal Continental Mediterranean climate. Twenty-eight populations from the Iberian Peninsula and Algeria were selected to encompass the climatic range of the Mediterranean lineage 2 (following Fernández et al. 2012) based on a preliminary PCA analysis including the main environmental variables (such as temperature and precipitation), and 17 populations from the three known species of *Carpetania* were chosen thorough their whole range. In total, 134 individuals of *Ap. trapezoides* and 85 individuals of *Carpetania* were sequenced using dGBS, using the restriction enzyme MsII, and an average of 2 million reads per individual were obtained. Information about the different environmental large-scale variables was obtained from publicly available layers. LFMMs (Latent factor Mixed Models) and the Sambada program was used to study the association between SNPs and environmental variables, taking into account the population structure, which could be a confounding factor. The SNPs under selection and those associated with environmental variability allowed the detection of genes that could be involved in adaptation to different environments. Those SNPs were mapped against a *de novo* assembled transcriptome to obtain candidate genes potentially involved in adaptation to the environment in earthworms. Our results provide insight into the evolutionary consequences of environmental changes in soil fauna and help to predict potential impacts and design suitable management and conservation strategies.

## Beyond morphology: species delimitation and phylogeography of the hypervariable earthworm species complex *Allolobophora molleri-moebii*.

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Constraints to soil life have imposed an optimized body model for key edaphic organisms such as earthworms, leading to a morphological stasis and cryptic species with great genetic diversity. However, the opposite phenomenon (high morphological variability displayed for the same genotype) may be occurring in earthworms such as the *Allolobophora molleri-moebii* species complex, resulting in the description of a multitude of species from an alleged single genetic lineage. To solve this taxonomic problem, specimens from 30 populations from Portugal, Spain and Morocco representing a wide range of variation in one morphological trait (clitellum position) have been collected. Clitellate individuals from each population were identified as one of the five species of the complex (*A. molleri*, *A. moebii*, *A. fernandae*, *A. monchicana* and *A. ophistosellata*) and their internal and external anatomy was studied. A set of seven molecular markers (COI, ND1, 16S, 12S, 28S, H3 and H4) were sequenced for representatives of each population. Finally, phylogenetic relationships were recovered by Bayesian inference and Maximum Likelihood analyses.

The results show the Moroccan population as the most phylogenetically distant, followed by the southern Spanish populations. However, phylogenetic clades recovered were generally inconsistent with morphological variability, recovering individuals of the different nominal species in the same clade on several occasions. Finally, COI pairwise genetic distances generally remained below 10% between populations, reaching the highest values between Iberian and Rifian populations, which highlights the north of Africa as the ancestral area of this species complex. This research would support previous literature that conceives the *A. molleri-moebii* species complex as a single hypervariable species. The distribution pattern of the five nominal species suggests an extreme phenomenon of phenotypic plasticity. This enhances the *A. molleri-moebii* complex as a great candidate for the study of the interactions between genotype, phenotype, and environment, and therefore, the adaptation of these animals to Climate Change. The phenotypic plasticity of this species complex opens a host of questions regarding the role of the clitellum in earthworm reproduction and the importance of this morphological feature in their taxonomy.

## Connectivity within and among sponges' populations in east Mediterranean Sea

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Establishing Marine-Protected-Areas (MPAs) and their networks, based on spatial connectivity of populations will enable maintaining the genetic diversity required for their existence. Thus, MPA communities will better cope with natural and anthropogenic-induced ecological changes, including climate change. In the last decade, mesophotic (90-130m deep) sponge grounds (MSG) were discovered in the East Mediterranean Sea along the Israeli coast. These habitats are oases of unparalleled local richness and diversity with over 100 sponge species, that serve as ecosystem-engineers and habitat-builders for more than 150 other invertebrate and fish species. New studies have shown that the MSGs' stable conditions facilitate their role as a refuge for shallow water species, and a potential source for larvae to colonize shallow habitats, where current conditions are harder. As such, some of these MSGs have been designated and promoted as MPAs. Due to anthropogenic threats to MSGs and shallower marine habitats, policymakers must be provided with scientific ecosystem analyses, to be considered upon MPAs planning. One of the difficulties in assessing the connectivity of sponge populations is that frequently the traditional methods discovered insufficient genetic variation. We used a novel molecular method (ddRADseq) that provides extensive information on genetic variation. We examined the genetic connectivity of *Axinella polypoides* and *Ircinica variabilis* in three depths (100, 40 and 25 m) between and within four sites along the Israeli coast: two are continuous-rocky habitats and two are patchy rocky habitats. In each location and depth, 15 specimens of each species were collected by either a Remotely-Operated-Vehicle, SCUBA, or technical diving. Preliminary results support the hypothesis of connectivity between *A. polypoides* populations in different sites that vary by depth and distance. Comparing these results with *I. variabilis*, which uses a different reproductive strategy, we will provide a more complete picture of the connectivity between these sites.

## Genetic Tools Reveal Invasive Eastern Mosquitofish Diet and Distribution in Spain: Insights for Endangered Toothcarp Conservation

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The Spanish toothcarp, *Aphanius iberus* and its sister species, the Baetican toothcarp (*Aphanius baeticus*) are two cyprinodontiforms endemic to the Iberian Peninsula which currently face extinction as a result of anthropogenic pressures including habitat destruction, the contamination of waterways, and the introduction of invasive species. The arrival of the invasive Eastern mosquitofish has particularly devastated the survival of both species, possibly due to a competition for resources, predation or both. In this preliminary study, we illuminate the promising potential of DNA metabarcoding techniques to uncover the negative impact of *G. holbrooki* on both *A. iberus* and *A. baeticus*. Through our genetic analyses, we provide compelling evidence that predation is a likely and ongoing phenomenon, further highlighting the urgency of conservation efforts. Moreover, we demonstrate the effectiveness of environmental DNA (eDNA) as a crucial tool for monitoring the current distribution range of all three species. This innovative approach offers valuable insights, enabling us to optimize conservation management strategies for the protection of *A. iberus* and *A. baeticus*. By integrating DNA metabarcoding and eDNA analyses into conservation practices, we can strive towards the preservation of these endangered toothcarps and their unique habitats in the Iberian Peninsula.

## Diversidad Genética y Filogenia de las especies del género *Squamaria Poelt* y sus Ficobiontes en Península Ibérica y Macaronesia

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El género *Squamaria*, introducido por Poelt en 1958, incluye especies de ascomicetos liquenizados distribuidos principalmente en el hemisferio norte, que se desarrollan en rocas calcáreas y silíceas, así como en suelos de arcilla y yeso. La mayoría de ellos tienen talos escuamulosos, a veces lobulados, saxícolas o terrestres, con abundantes y llamativos apotecios. Aunque las especies del género *Squamaria* son comunes en los ecosistemas mediterráneos, aún no se han llevado a cabo estudios exhaustivos sobre la diversidad genética y filogenética de los micobiontes y ficobiontes involucrados en estas simbiosis en Europa. En el presente estudio, se recolectaron talos de diferentes localidades en la península ibérica, tanto en su zona norte como en la oriental, en las Islas Baleares y en varios archipiélagos de la Macaronesia (Islas Canarias, Madeira y Cabo Verde), y se estudió la identidad y diversidad genética de los micobiontes y ficobiontes mediante enfoques filogenéticos utilizando datos moleculares de nrITS. La identidad de las microalgas también se corroboró con la observación de sus ultraestructuras mediante microscopía electrónica de transmisión. Además, se analizaron los patrones de interacción entre los diferentes linajes de micobiontes y ficobiontes, y se cuantificó la importancia relativa de la identidad genética de cada simbionte, el macroclima, la geografía y el sustrato en la determinación de la variación en la diversidad de micobiontes y ficobiontes a través de un estudio de partición de varianza. Los resultados revelaron una sorprendente diversidad genotípica en los micobiontes del género *Squamaria*, que se asocian con siete especies de microalgas clorofitas de los géneros *Asterochloris* Puymaly, *Vulcanochloris* Tschermak-Woess y *Trebouxia* Vancurová, Peksa, Nemcová & Škaloud. En las interacciones simbióticas, los patrones de especificidad variaron entre las diferentes especies, revelando, en la mayoría de los casos, una notable flexibilidad en la selección de ficobiontes. Sin embargo, la identidad genética del micobionte y del ficobionte resultó ser un factor clave en la diversidad y distribución de las especies de *Squamaria*, lo que habría permitido la colonización de ambientes bioclimáticamente diversos a través del establecimiento de simbiosis con especies de ficobiontes que podrían estar más adaptadas a las condiciones locales.

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## Continental African Euphorbiaceae: A model-clade approach to study The Odd Man Out pattern

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The “Odd-Man-Out” pattern [1] describes the observed lower plant diversity in continental tropical Africa compared to other tropical regions. Historical biodiversity loss linked to climate change is one of several explanations for the reported phenomenon. We examine long-standing hypotheses behind this pattern by adopting a model-clade approach that combines the in-depth knowledge of small-scale clade studies with the power of family-wide metanalysis, trying to reconcile opposing concerns in the field [2, 3].

We focus on 31 genera of the plant family Euphorbiaceae, which form 19 clades [4] and include approximately 551 species. Our workflow involves the revision of each genus' alpha-taxonomy and the compilation of information about traits linked to climate-change vulnerability like habit, geographic range and sexual system. Complementarily, we produce sequences for 431 low-copy nuclear genes per sample using the Euphorbia kit for HybSeq target sequencing to generate genome-wide phylogenies of each group of interest. The resulting life-history, biogeographic and phylogenomic information are then used to examine the specific mechanisms working at the clade-level and the general drivers acting clade-wide on Afrotropical lineages. To date, we have sampled more than 70% of the selected species, and generated new genomic data for all 33 focal genera. We have also produced the first extended phylogenomic time-tree for the African lineages of Euphorbiaceae including representatives of the three main subfamilies (Acalyphoideae, Crotonoideae y Euphorbioideae). For the genus *Croton* in particular, we have inferred a species tree for 45 of the 56 continental African species and 18 from Madagascar. This allowed us to recover the highly resolved phylogenetic relationships within this group of *Croton*, contrasting with previous results [5] that showed lack of resolution for the African and Malagasy lineages. Finally, our results support the polyphyly of the continental species [5] in relation to the Malagasy *Croton*.

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## Designing a novel biological water quality index: applying metabarcoding techniques using Arcellinida as a model

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The anthropogenic pressures are having an increased impact on freshwater ecosystems, even more under arid climates. Bioindication is the most widely used method nowadays for monitoring the environmental quality of these ecosystems. Several biomonitoring protocols have been developed and integrated into the Spanish legislation, like the IBMWP based on visual observation of macroinvertebrates. Nevertheless, these protocols suffer from laborious procedures and a deficiency in taxonomic precision, resulting in reduced sensitivity. Additionally, the majority of these protocols have been designed specifically for lotic systems and are ill-suited for lentic water bodies.

There is a need to explore new biological indicators that exist in all lentic environments, facilitating easy sampling and delivering sufficiently sensitive outcomes that are readily interpretable. Moreover, metabarcoding-based protocols offer desirable traits such as sensitivity, efficiency, cost-effectiveness, and reproducibility, while obviating the dependence on taxonomists' expertise.

Within this project, we are engaged in the development and implementation of a biomonitoring protocol that relies on the application of Arcellinida (=lobose testate amoebae) metabarcoding. Arcellinida, which are highly prevalent in inland water bodies, demonstrate a remarkable specialization in their ecological niche and exhibit rapid response times to environmental disturbances. These characteristics position Arcellinida as exceptional bioindicators for monitoring purposes.

The primary goal of this project is to create an innovative protocol for rapid and reliable monitoring of water quality in lentic systems, such as large river axes, natural lakes, brackish lagoons, and reservoirs. To achieve this, we will implement the developed protocol in diverse river basins located in Southern Spain, using metabarcoding to unveil the composition of Arcellinida communities. Additionally, we will use machine learning techniques to establish environmental quality indices, aiming to integrate this methodology into Spanish legislation to evaluate the environmental well-being of freshwater lentic ecosystems, which currently face significant threats.

## Deciphering the evolution of arthropod respiratory systems through bulk, single cell and spatial transcriptomics

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Animals change, adapt and are able to conquer new environments for their living, becoming masters of evolution with the passage of time, reshaping organs and habits. Understanding the genomic basis underlying such changes is a key question in evolutionary biology. Different species may come to the same solution (commonly referred to as convergent evolution) through innovation, tinkering, expansion or loss of their genetic material. A remarkable example is arthropod terrestrialization, which occurred at least 7 times in the history of Life on Earth. Our aim is to understand the genomic basis underlying functional homology of arthropod respiratory structures at the genomic levels across arthropod lineages that conquered land independently and that display different respiratory structures. By exploring different respiratory organs across arthropods (e.g., gills, trachea, book lungs and book gills), we aim to understand if genes in different respiratory structures have a common evolutionary origin. Specifically, we seek to elucidate whether "old" genes maintain consistent functionality across dissimilar respiratory structures or if "new" genes have been co-opted for convergent functions. For that, we will use a combination of bulk, single cell (scRNA-seq) and spatial transcriptomics of respiratory organs across arthropods. Through bulk transcriptomics, we will explore evolution of gene expression in different organs across arthropods, providing insights into the core genetic components involved in respiratory adaptations. scRNA-seq enables us to explore the cellular diversity and heterogeneity within arthropod respiratory organs, elucidating the cellular basis of macroevolutionary shifts in arthropod respiration. Spatial transcriptomics will offer an insight into spatial organization and cellular interactions within respiratory organs. Through these investigations, we aim to uncover the underlying evolutionary mechanisms that govern functional homology and diversification of arthropod respiratory structures. The outcomes of this study will significantly contribute to our understanding of the evolutionary processes that drive convergent evolution and shed light on the remarkable adaptations that have enabled arthropods to independently conquer terrestrial environments.

## Comparative microbiome analysis reveals differences between wild and captive populations of the Montseny Brook Newt (*Calotriton arnoldi*)

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The Montseny brook newt, *Calotriton arnoldi*, is an endemic species to the Montseny Natural Park in Catalonia, and it is currently classified as Critically Endangered by the IUCN. This species is divided into two geographically close but genetically isolated metapopulations situated in the Western and Eastern regions of the Tordera River Valley. Due to population decline and threats to its natural habitat, an ex-situ breeding program was initiated by the Generalitat de Catalunya in 2007.

Ensuring the survival of captive-bred individuals after reintroduction is a key part of the breeding program. For amphibians, it is crucial to ensure that the specimens' microbiome can protect them from other environmental microorganisms, particularly considering the global Chytridiomycosis pandemic caused by the fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*).

The main goal of this study was to characterize the microbiome of wild and captive specimens of *Calotriton arnoldi* for the Western and Eastern populations, to identify possible differences in microbiome composition, and determine their potential impact on the survival of captive-bred individuals upon reintroduction to their natural habitat.

The final database obtained consisted of 7,438 ASV obtained from 138 different samples. Results indicate that wild populations have significantly different microbiome composition, as do wild and captive-bred groups from the same population. Additionally, dissimilarities in microbiome variability were only found within populations between wild and captive-bred groups. In terms of composition, certain bacteria were identified as potential markers for both wild and captive environments such as a core wild ASV from the Cytophagales order which was absent in the captive groups. These results are relevant because enhancing microbiome variability could likely improve the survival prospects of reintroduced specimens. Thus, subjecting captive specimens to a more natural environment whilst in captivity or, alternatively, a soft-release procedure could potentially mitigate the absence of exposure to other bacteria and potential pathogens from their native environment.

## Introduced mesofauna in island soils: invasion patterns, determinants and resources for soil biodiversity conservation and island biosecurity (SoILinvaders)

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Soil constitutes one of the largest reservoirs of biodiversity on the planet, playing an essential role in the ecological processes on which the functioning of terrestrial ecosystems depends. In this sense, the edaphic mesofauna plays a crucial role, participating in fundamental processes such as carbon transformation, nutrient cycling, soil structuring, etc. However, in the soils, exotic species also threaten native biodiversity, and their synergy with climate change can cause dramatic effects on soils, which are even more critical on oceanic islands. Moreover, the soil is one of the least studied ecosystems, and this, added to the limitation of conventional approaches to the study of edaphic mesofauna, means that our understanding of the identity, distribution and richness, both native and non-native species, is also limited. We are unaware of the actual magnitude and main factors involved in introducing exotic species into the soils. In this sense, *SOILinvaders* is an innovative and multidisciplinary project which aims to characterize de magnitude, the drivers (abiotic and biotic) and the risk of species introduction in the soils of the Canary Islands, generating in turn essential tools and resources for the conservation of biodiversity and island biosecurity. All this, by applying last-generation molecular techniques such as whole organism community DNA metabarcoding (wocDNA MBC) to address three main objectives: (i) derive estimates for the magnitude of introduced species within oceanic islands soils by developing novel statistical approaches to identify introduced species from wocDNA MBC, (ii) identify major abiotic and biotic drivers of the introduction and establishment of introduced mesofauna in soils, and the generation of current and future invasion risk maps, and (iii) generate essential molecular and bioinformatic resources and tools for a long-term biomonitoring program on soil mesofauna and lay the foundations for a diagnostic insular biosecurity tool for soil and vegetation imports. Here we describe the *SOILinvaders* framework, present preliminary data obtained within the initial phase of the project and discuss further steps to provide quantitative measures of the prevalence of mesofauna introduction in soils.



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