Sessione
Approcci molecolari
nella zoologia contemporanea

Comunicazioni

Shark tales – the stories told by molecules

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Elasmobranchs (sharks and rays) are charismatic vertebrates that capture the public´s attention around the world. This ancient group of jawed vertebrates dates back 350 Myr, predating the dinosaurs, and surviving several mass extinction events. Currently, overfishing, habitat degradation and climate change have led to population depletion and to ˜1/4 of extant elasmobranchs being threatened, while ˜1/2 is Data Deficient. Indeed, studies based on direct observation of shark and ray populations are logistically challenging, costly and may induce individual mortality (e.g. fishing). Here I provide several examples of how molecular genetics, coupled to non-invasive or minimally invasive sampling, may provide important clues on elasmobranch biology, ecology, diversity and evolution, with important management and conservation implications. Specifically, molecular genetic studies have helped uncover important aspects of reproductive biology and behavior, such as the diversity of mating systems in elasmobranchs. On the other hand, population genetics and genomics have also helped clarify the main patterns and drivers of population structure of sharks and rays, and to identify the putative barriers to gene flow in different taxa. Molecular markers have also greatly improved species identification as well as detection of cryptic species, which are essential to accurate data collection and analysis. Technological advances in genetic data collection, namely next generation sequencing, have expanded the scope of the questions and the fraction of the genetic material available for interrogation. For instance, the number of high-quality, complete whole genomes for elasmobranchs have opened the way to comparative genomics and the opportunity to look at the evolution of jawed vertebrates and their unique features (e.g. an adaptive immune system) from the perspective of one of their most ancient and basal group – the Cartilaginous fish. Finally, the field of environmental DNA analysis offers the possibility to survey and monitor species diversity in time and space in a non-invasive way, which is particularly important in the case of the highly elusive and endangered elasmobranch taxa. I conclude with examples of knowledge gaps in elasmobranch ecology, diversity and evolution, where molecular tools can lead to major breakthroughs.

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

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Sawfishes represent one of the most critically endangered groups among elasmobranchs and have experienced local extinctions across numerous coastal regions worldwide. Contemporary conservation strategies increasingly highlight the importance of reconstructing historical changes in species distributions and geographic ranges. Within the Mediterranean, such reconstructions have indicated the historical presence of two sawfish species, presumed extinct since the 1970s, challenging earlier assumptions about their biogeographical range.
In this study, we investigated whether historical sawfish specimens preserved in various institutional and private collections could provide evidence of a Mediterranean origin. A total of 229 rostra were examined, including 28 specimens labelled as originating from the Mediterranean region. Most of these samples lacked precise taxonomic classification and robust collection metadata. We performed morphometric analyses alongside mitochondrial DNA sequencing (targeting partial COI and NADH2 regions) achieving the assignment of all rostra to four extant species: *Pristis zijsron* (104 specimens), *Anoxypristis cuspidata* (52), *P. pristis* (47), and *P. pectinata* (26). Among the rostra labelled as Mediterranean, we identified *P. zijsron* (9), *A. cuspidata* (8), *P. pristis* (6), and *P. pectinata* (5).
Following taxonomic identification, we implemented a preliminary isotopic approach to infer historical provenance, based on stable isotope analysis (^{13}C and ^{1}N) of rostral teeth and collagen-rich tissues from the rostra. Initial results revealed significant ^{13}C variation between specimens from the Red Sea and those from other documented localities. Furthermore, high ^{1}N values were observed in many A. cuspidata individuals, suggesting associations with nutrient-rich, upwelling-influenced coastal environments. The Mediterranean-labelled rostra remain tentative and require further validation through a comparison with historical baseline for elasmobranchs we are building up using the "P. Doderlein" osteological collection. These findings underscore the importance of integrating museum collections and stable isotope analyses to refine our understanding of sawfish natural history, establish historical population baselines, and could enhance global conservation planning.

Molecular tools to gain a deeper knowledge on Sardinian Chondrichthyan species

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

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

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

Mitochondrial genomes: a resource for Tardigrade phylogenetics

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

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

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

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

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

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

Comprehensive DNA barcoding of Italian birds

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

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

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

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

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

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

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

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

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

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

We present case studies highlighting various eDNA sampling strategies:

Five eDNA sampling systems (active and passive) were tested in a controlled environment. Active samplers yielded more DNA and detected all elasmobranch species; passive tools were less efficient.

Passive samplers deployed with deep-sea longlines detected 78% of the species captured and uncovered additional pelagic and mesopelagic taxa missed by traditional methods.

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

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

All samples were processed by eDNA metabarcoding with an elasmobranch-specific marker. Taxonomic assignment was based on a custom 12S reference database that incorporated newly generated and curated public sequences. The accuracy was improved by increasing taxonomic

coverage and integrating results from 12S, COI and NADH2 markers. Over 15% of public sequences were found to be mislabelled. Our custom database now allows reliable identification of up to 90% of Mediterranean cartilaginous fish species.
These findings confirm eDNA as a powerful tool for comprehensive marine biodiversity assessments. While challenges remain, especially in standardizing methods and interpreting data, addressing these will enhance eDNA's role as a cost-effective, large-scale tool, laying the foundation for a stronger contribution to informed conservation strategies and policy development.

Small basin, big data: eDNA metabarcoding tracks ve 1 rtebrate diversity and distribution

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

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

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

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

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Soil is a fundamental environment hosting a high biodiversity and enclosing numerous microhabitats. Multiple studies have proved that the adoption of more sustainable practices in crop cultivation results in improved soil biodiversity conservation, whereas conventional agronomic practices can have detrimental impacts on soil attributes. Besides, rice is a major staple food and at the same time, the landscape heterogeneity within its agroecosystem is of fundamental need for several different organisms linked permanently or temporarily to water.
In this study, we used an environmental DNA metabarcoding approach targeting two different genes, the 18S rRNA and the cytochrome C oxidase subunit I (COI), aiming to assess the changes in the protist and metazoan soil communities and their functional diversity under three different rice cultivation managements (i.e. agroforestry, organic and conventional), and along four stages of the rice growth cycle (i.e. basal, vegetative, flowering and maturation phases).
Results showed that the most abundant phyla in the rice paddy soil were Cercozoa and Ciliophora for protists, and Annelida, Nematoda and Arthropoda for metazoan. In particular, Cercozoa were abundant in the agroforestry cropping system, while Ciliophora showed higher abundance in control cover crop field. Annelida were more abundant in the conventional cultivation regime, while both Nematoda and Arthropoda were less abundant, with a significant increase in the organic and agroforestry regimes. Considering the taxonomical and functional diversities, slight differences among treatments were identified both in protists and in metazoans because of the combined effect of agricultural management and the succession of drying-flooding phases during the growing season.
The community's beta diversity described a positive effect of the organic and agroforestry cropping systems, highly dissimilar from the community found in the conventional rice field. Soil physical-chemical properties did not differ significantly from one treatment to another. This study broadens our understanding of the effects of agricultural practices on the biodiversity inhabiting the soil in rice agroecosystems, highlighting the positive impact of organic and agroforestry management as suitable environments for the rice soil biocenosis. It also contributes to emphasizing the importance of soil biodiversity conservation and the benefits of redesigning agricultural practices.

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

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

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

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

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

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

Evolution of desiccation related proteins in the tardigrade family Ramazzottiidae

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Tardigrades are a phylum of micrometazoan known for their ability to survive desiccation (anhydrobiosis). Research has focused on unveiling the molecular mechanisms behind their survival abilities, with a great interest in their biotechnological potential. In particular, by studying the model species *Ramazzottius varieornatus* and *Hypsibius exemplaris*, multiple tardigrade-specific gene families contributing to anhydrobiosis have been discovered. While almost all studies focused on the above-mentioned model species, evolutionary and comparative analyses of anhydrobiosis gene families are scarce. Our study is aimed to fill this gap by providing a comparative analysis of different anhydrobiosis-related gene families in the tardigrade family Ramazzottiidae.
We sequenced the genomes of 11 individuals belonging to 10 species from the family Ramazzottiidae (genera *Hebesuncus*, *Cryoconicus* and *Ramazzottius*). Single animals were used for Whole Genome Amplification coupled with Nanopore long-read sequencing. Assemblies were annotated ab initio and the relevant genes (Cytoplasmic, secreted and mitochondrial abundant heat-soluble proteins [CAHS, SAHS, MAHS] and Damage suppressor [Dsup]) were identified by blasting against a reference database of known sequences from those gene families. Genes sequences were manually curated and phylogenies were constructed using their aminoacidic sequences.
The assemblies ranged from about 50 to 100 Mb and were of good quality, despite a big range in their statistics (mean coverage range 55 – 280X, N50 range 35547 – 659307). The MAHS and Dsup were found with only one copy per species, while CAHS and SAHS experienced extensive duplications, at many points in their evolutionary history. In particular, the CAHS6 family split into four subfamilies after *Cryoconicus* + *Ramazzottius* diverged from *Hebesuncus*.
Our results indicate the WGA + Nanopore sequencing to be an extremely promising and easy approach to sequence tardigrade genomes. The CAHS and SAHS are highly dynamic genes that were involved in multiple duplication events even at shallow phylogenetic depths, which could indicate their importance in adaptations to local conditions experienced by different species. The cataloguing of different sequence variant of proteins of biotechnological interest, like the ones studied here, can help improve our knowledge about which sequence features are conserved and important for their function.

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

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

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

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

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

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

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

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

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

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

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

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

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

New insights into the probing behaviour of *Aedes mosquitoes*

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After landing on the skin of a mammalian host, female mosquitoes of the *Aedes* genus perform intradermal probing, or rather start salivating and piercing the skin moving their mouthparts in a stereotypical way. While probing, mosquitoes spot blood vessels to acquire and digest blood and complete egg development. Fastening intradermal probing is crucial to shorten blood feeding, a necessary but risky step in mosquitoes' life.
We have characterised the role of Labrum Interacting Protein of the Saliva (LIPS)-2 in modulating intradermal probing in the tiger mosquito *Aedes albopictus* (Skuse, 1895). LIPS-2 is a female-specific saliva-enriched protein: knocking down its expression in female mosquitoes increased their probing time while blood feeding on human volunteers. After secretion, LIPS-2 is reabsorbed at the tip of the labrum, the mouthpart forming the food channel, where it binds Cuticular protein (Cp)19. The binding of LIPS-2 leads to a modification of the height of labral ridges, two cuticular structures located at the tip of the labrum hosting labral ridges receptors (lrr), which have been previously suggested to be proprioceptive. We have hypothesised that the Cp19:LIPS-2 interaction mediates proprioception of the labrum during probing, helping to control the movements of this stylet while searching for a blood vessel.
We have recently exploited genetic engineering, advanced imaging techniques and quantitative behavioural experiments to understand the molecular mechanisms outstanding LIPS-2 function. Aedes albopictus LIPS-2 knockout and control strains were generated in collaboration with Dr. Papathanos and their probing and feeding times, together with the amount of acquired blood, were evaluated in feeding experiments on human volunteers. *In vitro* feeding experiments were also performed exploiting the BiteOscope, which allows high resolution and high throughput analysis of mosquito feeding by automatic extraction of behavioural statistics from image sequences. Finally, scanning electron microscopy (EM) and volume EM were carried out on the labrum, with a particular focus on lrr.
Behavioural experiments were useful to explore the role of LIPS-2 in proprioception and chemosensation, while imaging analyses provided data about the morphological organisation of lrr. These analyses represent a base for a deeper and future investigation of the function of lrr in perception and of Cp19:LIPS-2 interaction in the modulation of intradermal probing.

Interspecies comparative spatial transcriptomic of mosquito male accessory glands

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In insects, seminal fluid proteins (SFPs) are secreted by the male accessory glands (MAGs) and play a pivotal role in reproductive success. These proteins influence female behaviour - most notably by stimulating egg-laying and reducing receptivity to further mating. SFP genes evolve rapidly, likely driven by post-copulatory sexual selection arising from sperm competition and cryptic female choice.
Despite their significance, the spatial organization of gene expression within the MAG remains poorly understood, with existing studies limited to a few species and selected genes. In this study, we employ spatial transcriptomics to map and compare gene expression patterns across the MAGs of five mosquito species: *Aedes albopictus*, *Aedes aegypti*, *Aedes koreicus*, *Culex pipiens*, and *Anopheles stephensi*. These species were selected based on their phylogenetic relationships, encompassing both closely and distantly related lineages. MAGs were dissected, embedded in OCT compound, and cryosectioned using a cryostat. Tissue sections were placed onto Curio Seeker tiles, which consist of a monolayer of spatially indexed beads. Following tissue placement, RNA transcripts were captured by the beads, and Illumina libraries were prepared and sequenced. The resulting sequence data were analysed using the Seeker bioinformatics pipeline and the R programming environment.
Our analysis reveals spatially distinct zones of transcriptional activity within the MAGs, corresponding to functional regions associated with seminal fluid protein (SFP) synthesis, secretion, and structural maintenance. A consistent anatomical organization emerged across species, with the presence of two main regions—an anterior and a posterior domain—observed in all examined MAGs. However, gene expression profiles showed low conservation across species, indicating substantial divergence.
Comparative analysis identified both conserved and species-specific transcriptional patterns, suggesting evolutionary differentiation in reproductive strategies and post-mating interactions. This study presents the first spatially resolved transcriptomic atlas of mosquito MAGs and the first cross-species spatial comparison, offering new insights into the molecular architecture of male reproductive tissues and the evolutionary dynamics of sexual selection in vector mosquitoes.

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

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

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

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

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

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

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

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

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

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

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

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

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