Sessione
Approcci molecolari
nella zoologia contemporanea

Poster

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Tentacular barcodes: building reference libraries for Mediterranean cephalopods

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Species delimitation by traditional morphological methods is challenging in cephalopods due to their flexible bodies, changeable pigment traits, and sometimes high morphological homoplasy. Additionally, samples could be heavily damaged during collection making the correct identification of the specimens at species level more difficult. Correctly identifying the species is critical to evaluate their distribution, abundance and role in the ecosystem. This is particularly relevant for cephalopods that play crucial roles in marine ecosystems, acting as both predators and prey for apex predators, thereby contributing to the distribution of energy and nutrients across the food web.
In this context, DNA barcoding offers a useful and powerful tool for reliable and rapid taxonomic assignment. Despite the important possibilities, this approach is partially limited by the insufficient coverage of reference sequence databases and the inaccuracy in their taxonomic labels.
With the present work, we aim to produce a curated multimarker reference library for the 72 cephalopod species inhabiting the Mediterranean Sea. Moreover, analysing public sequences the robustness, reliability and completeness of the online databases (NCBI and BOLD) are evaluated. Along with new 'referenced' sequences for the Mediterranean cephalopods, our results provide clear evidence of errors in public repositories such as: a) mislabeling and/or misidentification, b) old nomenclature and/or data not updated, c) the presence of many possible new species not yet described and d) clades which require further analysis to solve taxonomic uncertainties. In particular, the obtained results suggest careful scrutiny and manual corrections of publically obtained sequences by experts prior to their use.
Overall, our results highlight the current limits in studies on cephalopods using barcoding and/or metabarcoding approaches, mainly linked to the low species coverage rate of available sequences, taxonomic uncertainties and the relative low reliability of public databases. Further work is needed in order to complete and improve the curated reference libraries, basic requirements in molecular studies.

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

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

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

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

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

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

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

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

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Effective fisheries management and conservation planning depend on accurate species identification, particularly for taxa vulnerable to direct exploitation or incidental capture. Mitochondrial DNA barcoding offers a reliable tool for species identification, even among morphologically similar or cryptic taxa. Aotearoa New Zealand supports a remarkably diverse chondrichthyan (sharks, rays, and chimaeras) assemblage, hosting over 10% of the global chondrichthyan diversity. About 20% of species are endemic to the region. Novel species continue to be described regularly, and taxonomic uncertainties require further investigation. This knowledge gap is reflected in the fact that over one-third of the New Zealand chondrichthyans are assessed as regionally Data Deficient. For this reason, accurate species identification is essential for developing effective conservation and management strategies.

In the present study, we applied a DNA barcoding approach to identify chondrichthyan species caught on Chatham Rise (New Zealand) during a scientific bottom trawl survey. Each specimen was analysed using COI and NADH2 mitochondrial DNA markers.

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

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

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Small-sized freshwater species with little or no direct economic value are often threatened yet poorly understood from an ecological perspective. *Lampetra zanandreai* (Vladykov, 1955) is a non-parasitic, strictly freshwater lamprey endemic to the ancient Po basin and threatened with extinction. Apart from some general information on its biology and life cycle, much of its ecology, including its feeding habits, remains unknown. This study aimed to investigate the feeding habits of the Po brook lamprey using a molecular approach.
Twenty-seven specimens and sediment samples were collected from two different streams in the upper Po basin (NW Italy) and analysed. The content of the digestive tracts were collected in the laboratory and preserved in absolute ethanol. Species composition of these contents was investigated through DNA metabarcoding using the primer set 1391f/EukBr targeting the V9 hypervariable region of eukaryotic 18S rDNA. Sediment samples were also analysed using the same metabarcoding protocol to identify potential trophic sources.
Taxa other than lamprey were found in 15 out of 27 individuals. The most frequently found taxon in the digestive tract contents was Ciliophora (found in 9 individuals), followed by Euglenozoa (5 individuals), Chlorophyta and Nematoda (2 individuals each), Cryptomycota and Mollusca (1 individual each). All the taxa were also found in the sediment with the exception of Mollusca.
This is the first molecular assessment of the diet of *L. zanandreai* and provides new insights into the trophic ecology of this endemic, threatened and neglected species. A more detailed analysis using additional primers is underway. Understanding the ecological requirements and needs of different species is essential for effective conservation strategies, and diet is a key ecological parameter.

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

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Soil ecosystems harbour a rich biodiversity and a complex community of different animal taxa known as edaphic fauna. Among these, microarthropods play a crucial role in nutrient cycling and soil transformation. Many studies focus on investigating this biodiversity and unravelling the relationship among different taxa; unfortunately, there are some animal groups for which the species identification is harder for the lack of morphological and molecular data. One of these groups is Pauropoda (Myriapoda), microarthropods that live in the first layers of soil. Their main taxonomic features are the segmented antennae with three flagella and an antennal globulus, and the morphology of the anal plate. The "taxonomic impediment" is particularly high for Pauropoda since published data mostly derive from a single author (Ulf Sheller; now deceased), molecular data are scarce, and no dichotomous key is available to enable a reliable species identification.
This research aims to improve morphological and molecular approaches for the species identification to expand the knowledge on the diversity of Italian Pauropoda using an integrative approach.
Sampling took place in nine localities of Emilia Romagna and Tuscany. Before undergoing molecular analyses, specimens were fixed and conserved in ethanol 70°-100° at -20°C, mounted on slides with glycerol and ethanol 70°, and observed with optical microscope up to 40x for species identification and to take pictures of the main taxonomic features. In addition, some specimens were used to observe taxonomic features with Scanning Electron Microscope (SEM).
A non-destructive DNA extraction was performed to ensure the retrieving of the carcass, which was subsequently mounted on slides with solution Gisin A (lactic acid, glycerol and formalin), as voucher specimen. Ultimately, amplification of COI and 18S genes was performed to obtain DNA sequences used for taxa identification and phylogenetic analyses.
Some species were recorded for the first time in Italy, expanding their known distribution range. Also, the integration of classical taxonomy with non-destructive DNA extraction and SEM imaging offers a promising framework for species-level identification.
Further analyses and broader sampling will be essential to clarify phylogenetic relationships and improve the taxonomic resolution of this group.
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Integrative taxonomy towards NIS: the case of *Pseudodiaptomus spp.* (Copepoda, Calanoida) from the Mar Piccolo of Taranto (Ionian Sea)

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

Uncovering Mediterranean mesophotic corals through integrative taxonomy

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

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

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

Genetic insights into *Salmo letnica* morphotypes in Lake Ohrid

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

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

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

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

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

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

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

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

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

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

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

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Sharks of the genus *Oxynotus* (Chondrichthyes: Oxynotidae) are rare and poorly known benthic elasmobranchs, distributed in deep marine environments worldwide. Among them, the angular roughshark *Oxynotus centrina* (Linnaeus, 1758) is the only species of the genus reported in the Mediterranean Sea. Due to its biological traits, it is highly vulnerable to fishing pressures. Moreover, having experienced in the recent past a severe population decline in this area, it has been listed as Critically Endangered in the IUCN Red List.
Through the analysis of two mitochondrial markers (COI and NADH2) widely used in elasmobranch studies, this work aims to investigate the intraspecific diversity and population connectivity of the species. Furthermore, the newly produced sequences were used to investigate the phylogenetic relationships among the species of the genus *Oxynotus*. To reach this scope, a total of 80 tissue samples were collected in Sardinian waters and from other Mediterranean areas, and the sequences newly produced here were compared with those already available in online databases.
Preliminary results based on the concatenated dataset (COI+NADH2) indicate very low levels of genetic variability and a low number of haplotypes in *O. centrina*, suggesting a potential high connectivity among areas. Phylogenetic trees show a close relationship between the sailfin roughshark *O. paradoxus* Frade, 1929 and the prickly dogfish *O. bruniensis* (Ogilby, 1893), clearly separated from *O. centrina*. In addition, COI sequences suggest the occurrence of possible cryptic species and mislabelling/misidentification within the genus.
Our results provide new genetic data for this poorly known species and lay the basis for future research that should encompass all the species included in the genus, presently lacking molecular characterization. Given the vulnerability of deep-sea species to environmental changes and human impacts, the implementation of baseline information is awaited to be provided for additional species, as it is essential for developing informed management and protection plans.

Avian flu in marine mammals: a phylodynamic and prevalence analysis

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

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

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

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

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

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

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

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

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

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Callinectes sapidus (Rathbun, 1896), native to the western Atlantic Ocean and the Gulf of Mexico, has become a prominent invasive species in the Mediterranean over the past decade. Its success is largely attributed to its high adaptability and aggressive predatory behaviour, which have led to its classification as a highly invasive species. Since the early 20th century, it has spread into the Mediterranean Sea, raising concerns about its potential ecological and economic impacts.
The blue crab thrives in both marine and brackish environments, with its life cycle closely tied to variations in salinity and temperature. Juveniles and adults typically inhabit estuarine and coastal waters, where salinity levels fluctuate. For reproduction, females migrate to high-salinity areas to spawn, and larvae develop in these saltier environments before dispersing into lower-salinity estuaries.
In this context, the ANNOTATE project monitors *C. sapidus* through environmental DNA (eDNA) in river tributaries flowing into the Marano and Grado Lagoon (16,364 ha, Northern Adriatic). Although the species has been established in the lagoon since the early 2000s, this study investigates its ongoing expansion into connected freshwater environments, with sampling conducted at 48 sites.
Surface water from six rivers was sampled monthly during summer and fall at two sites per river. Water parameters were recorded, including temperature, salinity, conductivity, dissolved oxygen, and pH. eDNA was extracted from the water samples and analyzed using a species-specific COI barcoding assay based on hydrolysis probe chemistry in qPCR to detect the presence/absence of *C. sapidus*.
Initial results revealed considerable variation in temperature and salinity across both rivers and sampling points. Mean temperature ranged from 17.7°C to 21.5°C, while salinity values varied from 3.5 $\times$ 10 ppm to 238 ppm. *C. sapidus* was detected in all surveyed tributaries, including the northernmost sampling points, located approximately 20km from the river mouths.
Overall, these findings confirm the species' capacity to tolerate a broad range of environmental conditions supporting its continued ecological expansion. Moreover, this study highlighted the effectiveness of eDNA as a powerful tool for detecting the distribution of invasive species in aquatic ecosystems.
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Safeguarding a freshwater sentinel: eDNA-Based conservation of *Austropotamobius pallipes* across borders

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

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

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

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

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

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