

2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

in collaboration with



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



UNIVERSITÀ
POLITECNICA
DELLE MARCHE



GAMES project

**GAMES: Genetica di popolazione e adattamento locale della vongola del
Mar Mediterraneo *Chamelea gallina* per azioni di gestione e
conservazione (PRIN 2022)**

SCIENZE
DISVA - DIPARTIMENTO DI
SCIENZE DELLA VITA E DELL'AMBIENTE





2nd Symposium
Environmental **cHANGES**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

Organizing Committee



Maria Assunta Biscotti, Polytechnic University of Marche



Adriana Canapa, Polytechnic University of Marche



Marco Barucca, Polytechnic University of Marche



Federica Carducci, Polytechnic University of Marche



Elisa Carotti, Polytechnic University of Marche



2nd Symposium
Environmental cHANGes,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

Scientific Committee



Stefania Gorbi, Polytechnic University of Marche



Maura Benedetti, Polytechnic University of Marche



Giorgia Gioacchini, Polytechnic University of Marche



Eva Šatović Vukšić, Rudjer Boskovic Institute



2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

Enhance

The symposium is organized in two sessions “conservation biology” and “repetitive DNA” in animal species. The former aims to understand how genetic biodiversity is crucial for species resilience; the latter focuses on the role of “repetitive DNA” in shaping genomes and regulatory networks to allow species adaptation.

Environmental changes are strongly impacting on our Planet causing loss of biological diversity. Many species have reduced population size and are at risk of extinction. It is now recognized the need to manage and conserve biodiversity resources and geneticists are playing an increasingly important role in this field identifying and monitoring the genetic variability. Indeed, genetic diversity is central to the long-term survival of any species since high levels of genetic variability are determinants of future population/species evolution, adaptation, and resilience. Several molecular markers have been adopted for DNA analyses and recently there is an increasing use of whole-genome sequence given its powerful potential of assessment. All these issues will be part of the session on “conservation biology”.

The second session will be dedicated to repetitive DNA, one of the most fascinating component of eukaryotic genome. For a long time, it was considered junk DNA. Nowadays, it is recognized its pivotal role in shaping genomes and regulatory networks allowing species adaptation. Moreover, repetitive sequences create genetic biodiversity that is also crucial for species resilience.



2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

Keynote Speakers



Francesco Ferretti, Department of Fish and Wildlife Conservation, Virginia Tech



Matteo Zarantoniello, Polytechnic University of Marche



Francisco De Menezes Cavalcante Sassi, Southwest University, Chongqing, China and Australian Amphibian and Reptile Genomics Initiative (AusARG).



2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

CONSERVATION BIOLOGY

Oral presentation,

Chairs: Giorgia Gioacchini, Federica Carducci, Stefania Gorbi, and Maura Benedetti



Revealing the Role of Toll-Like Receptor genes in Response to Rising Sea Water Temperature in the Antarctic fish *Trematomus bernacchii*

Shaghayegh Kholdihaghighi^{1,2}, Elisabetta Piva¹, Alessia Ametrano³, Sara Pacchini¹, Laura Drago¹, Martina Cortese¹, Paola Irato¹, Maria Rosaria Coscia³ and Gianfranco Santovito¹

¹Department of Biology, University of Padova, Padova, Italy

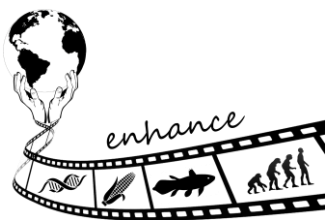
²Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Venice, Italy

³Institute of Biochemistry and Cell Biology, National Research Council of Italy, Naples, Italy

Abstract

The increasing rate of climate change, especially in polar areas, has driven an urgent need to understand how Antarctic fish cope with rising temperatures. Of particular interest is the effect on their immune system, as temperature increases can disrupt homeostasis and make organisms more susceptible to infections and diseases. Fish primarily rely on innate immunity, mediated by pattern recognition receptors (PRRs) such as toll-like receptors (TLRs). For the first time, this study analyzed the effect of temperature fluctuations on the expression of TLR genes in the Antarctic teleost *Trematomus bernacchii*. To this aim, fish specimens were exposed to acute thermal stress, mimicking short-term temperature increases due to climate change, up to +3 °C above the baseline environmental value of approximately –2.0 °C. The mRNA expression of chosen TLR genes (*tlr2* and *tlr9*), previously sequenced in two Antarctic fish species, was analyzed in the spleen and liver. The results underline changes in TLR transcript levels following thermal stress and confirm the species' sensitivity to such conditions. Furthermore, the genes are differentially expressed in the two organs analyzed, suggesting an organ-specific response. To provide a more comprehensive sight of the molecular response to elevation of the temperature, the study also assessed the expression of antioxidant genes from the peroxiredoxin family (*prdx3* and *prdx5*) in the same tissues. Significantly, it aims to identify potential relationships between immune gene modulation and peroxiredoxin activity, offering insight into the crosstalk between oxidative stress and immune responses under climate stress. These preliminary findings suggest the relative susceptibility of Antarctic fish to climate change, with important implications for ecosystem resilience and future conservation strategies.

Acknowledgements: This work was funded by the Italian National Program for Antarctic Research (PNRA), project No. 2018/B2Z1.01



Coping strategies of the Antarctic fish *Trematomus bernacchii* to marine heatwaves simulation

Elisabetta Piva¹, Alessia Ametrano², Sara Pacchini¹, Shaghayegh Kholdihaghighi^{1,3}, Laura Drago¹, Martina Cortese¹, Paola Irato¹, Maria Rosaria Coscia² and Gianfranco Santovito¹.

¹Department of Biology, University of Padova, Padova, Italy

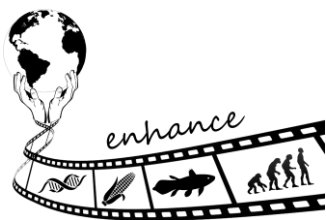
²Institute of Biochemistry and Cell Biology, National Research Council of Italy, Naples, Italy

³Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Venice, Italy

Abstract

Consequences of climate change are profoundly disrupting Antarctic oceanic ecosystems, with marine heatwaves (MHWs) appearing as critical stressors. Unlike gradual warming, MHWs are localized, short-term temperature spikes that challenge thermally sensitive Notothenioid fish, highly adapted to cold (about -1,8°C), stable environments. Characterized by limited thermal tolerance and slow metabolic rates, notothenioids could be particularly vulnerable to these acute warming events, which can disrupt physiological and immune homeostasis. This study investigates the molecular and physiological responses of a notothenioid species, *Trematomus bernacchii*, to thermal stress, focusing on how immune and antioxidant systems adjust to MHW-like events. Adult fish were sampled from the Ross Sea at Baia Terra Nova (74°42'S, 164°7'E) at depths of 60 to 100 meters and exposed to a gradual temperature increase from 0°C to +3°C, simulating acute warming scenarios. We continuously checked core body temperature and heart rate over a 15-day period using modern implantable bio-loggers, revealing a statistically significant positive correlation between internal temperature and cardiac activity, with heart rate increasing by approximately 25% for each °C rise. We analyzed by qPCR the expression levels of key genes of innate and adaptive immunity, in liver and spleen. Our findings offer novel insights into the ability of Antarctic fish to keep immune defenses while coping with thermal stress. Preliminary results, combined with data obtained by analyzing key antioxidant system-related genes in the same organs, contribute to a deeper understanding of the acclimation mechanisms of Antarctic fishes. By shedding light on the challenges faced by *T. bernacchii* during MHWs, this study advances predictions on the resilience of Antarctic fish fauna in the face of accelerating climate change scenarios.

Acknowledgements: This work was funded by the Italian National Program for Antarctic Research (PNRA), project No. 2018/B2Z1.01 and supported in part by the National Antarctic Research Programme (PNRA) (Project n° PNRA0000059-DIMANT)



Exploring the role of stress granules in *Squalius cephalus* during PFAS-induced oxidative stress

Laura Drago¹, Sara Pacchini¹, Martina Cortese¹, Giacomo Vanzan¹, Elisabetta Piva¹, Shaghayegh Kholdihaghi^{1,2}, Paola Irato¹, Andrea Bottacin-Busolin³, Andrea Marion³, Gianfranco Santovito¹

¹ Department of Biology, Università degli Studi di Padova, Padova, Italy

² Department of Environmental Sciences, Informatics and Statistics, Università Ca' Foscari di Venezia, Venezia, Italy

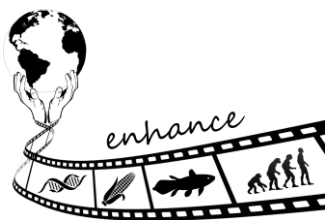
³ Department of Industrial Engineering, Università degli Studi di Padova, Padova, Italy

Abstract

Squalius cephalus, a fish endemic to rivers in the Veneto region, has recently been used as an experimental model to assess the effects of exposure to perfluoroalkylated substances (PFAS) and related physiological responses. Previous studies have suggested that the responses against PFAS-induced oxidative stress are probably regulated by the assembly/disassembly of particular cytoplasmic complexes of proteins and mRNAs called stress granules (SGs). SG formation occurs with the overexpression of mRNA binding proteins, such as TIAR, G3BP and TTP, which temporarily block the translation of specific mRNAs stored inside SGs. In this way, eukaryotic cells can rapidly activate protein synthesis in the presence of acute/chronic stress.

In this study, we aimed to assess whether SGs play a role in the post-transcriptional regulation of the expression of the antioxidant enzymes glutathione peroxidase (GPx) and catalase (CAT). In the liver and the caudal kidney of *S. cephalus*, which are organs known to be involved in xenobiotic accumulation and detoxification processes, the transcription levels of SG-related genes, i.e. *tiar*, *g3bp*, and *ttp*, and genes for antioxidant enzymes, i.e. *gpx1*, *gpx4*, and *cat*, were quantified by qRT-PCR. Biochemical assays evaluated GPx and CAT tissue activities. In addition, *in situ* hybridization and immunohistochemistry experiments were carried out to better assess the dynamic formation of SGs.

The obtained results allowed us to start understanding the possible interaction between SGs and the antioxidant system and the potential of SGs as biomarkers for environmental monitoring.



Priming techniques as a tool to ameliorate bivalves' tolerance to environmental stressors.

Deborah Cesaroni¹, Alessandro Nardi¹, Giovanna Monticelli², Luca Peruzza², Ilaria Bernardini², Giulia Dalla Rovere², Valentina Tavalazzi¹, Marica Mezzelani¹, Massimo Milan², Maura Benedetti¹

¹ Department of Life and Environmental Sciences- Polytechnic University of Marche, Ancona, Italy

² Department of Comparative Biomedicine and Food Science, University of Padua, Padua, Italy

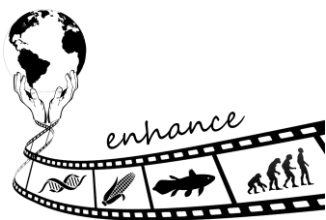
Abstract

In the last decades, climate change related stressors as marine heatwaves and acidification coupled to anthropogenic pollution have strongly affected marine environment, threatened marine biodiversity and also caused severe impacts to farmed and natural bivalves.

Among these, Manila clam *Ruditapes philippinarum*, has been subjected to several mortality events as a consequence of harsh environmental conditions, which resulted in socio-economic losses, being this species of high economic value in aquaculture, especially in Northern Italy.

With the aim of improving clam performance under multiple stressful conditions, we explored the feasibility and efficiency of chemical and thermal priming strategies. These are emerging approaches consisting in triggering the stress-response mechanisms in organisms with a moderate bout of stressful conditions and thus preparing them towards harsher ones.

To this end two laboratory experiments were carried out to (i) select the most suitable priming conditions and (ii) test the efficiency of priming in response to a combination of chemical and thermal stress, using 2µg/L of perfluorooctanoic acid (PFOA) and simulating a marine heatwave scenario. Digestive gland and gill tissues were collected to analyze a wide panel of biological responses, including immunocyte alterations, cholinergic function, antioxidant defences, peroxidation products, and lipid metabolism. Obtained results highlighted chemical priming as promising approach to promote transient tolerance to cultured bivalves under environmental stress scenarios, opening new opportunities for biodiversity conservation and sustainable bivalve aquaculture management.



Precision-Cut Tissue Slices (PCTS) as a sustainable and innovative *ex vivo* approach to assess the impact of emerging pollutants

Michela Panni¹, Marica Mezzelani¹, Maria Elisa Giuliani², Paola Nisi Cerioni¹, Alessandro Nardi¹⁻³, Maura Benedetti¹, Ernesto Mollo⁴, Francesco Regoli¹⁻³, Stefania Gorbì¹⁻³

¹ Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche (60131), Ancona, Italy

² Advanced Technology Center for Aging Research and Geriatric Mouse Clinic, IRCCS INRCA, 60121 Ancona, Italy

³ NBFC, National Biodiversity Future Center, Palermo, Italy

⁴ Institute of Biomolecular Chemistry, National Research Council, 80078 Pozzuoli, NA, Italy

Abstract

Environmental stressors, including climate change and anthropogenic pollution, pose significant threats to marine biodiversity and ecosystem integrity. To minimize animal use in toxicological research and support conservation efforts, the adoption of biological models aligned with the 3Rs principles (Replacement, Reduction, Refinement) is crucial. Precision-Cut Tissue Slices (PCTS) offer an innovative *ex vivo* approach that preserves tissue architecture and cell–cell interactions, thereby providing a physiologically relevant system to assess molecular and biochemical responses. By reducing reliance on *in vivo* experiments, PCTS contribute to more sustainable research practices and aid in the preservation of natural populations.

In this study, the *ex vivo* PCTS technique was applied to evaluate the effects of emerging pollutants using the Mediterranean mussel *Mytilus galloprovincialis* as a model organism. Slices of mussels digestive gland were exposed to different concentrations of environmental pharmaceuticals to investigate the onset of cellular, biochemical, and molecular alterations affecting immune system, biotransformation pathway, lipid metabolisms and redox homeostasis. Obtained results allowed to clarify pharmaceuticals' mechanisms of toxicity, further validating this approach as a suitable tool for ecotoxicological studies and to reduce the use of organisms in experimental research.



Examining the liver's role in regulating the circadian rhythm in female swordfish

Giulia Chemello¹⁻², Sara Filippi¹, Chiara Cardillo¹, Kevin De Simone¹, Erica Trotta¹⁻², Oiana Carnevali¹⁻², Giorgia Gioacchini¹⁻²

¹ Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy

² Istituto Nazionale Biostrutture e Biosistemi, Consorzio Interuniversitario (INBB), Rome, Italy

Abstract

Circadian rhythms regulate nearly all physiological and metabolic processes, allowing organisms to adapt to environmental changes. However, little is known about these pathways in large marine species like the swordfish (*Xiphias gladius*). During the reproductive season, mature females are more susceptible to environmental stressors due to the energy demands of reproduction, compromising their immune function and detoxification capacity. During reproduction, mature and immature females show distinct gonadal expression patterns of melatonin receptors and opsin genes. These seasonal variations may influence swordfish biology beyond reproduction, affecting metabolism and stress responses, although the exact mechanisms remain unclear. The present study investigated the presence of hepatic peripheral rhythms related to melatonin and opsin activity in mature and immature female swordfish. In 2019, female specimens were collected from the central Adriatic Sea in collaboration with fishermen of Fano fleet (Italy). Each specimen was weighed, and sexual maturity was determined by histology. The presence of melanomacrophages, melanomacrophage centers, and lipid was also evaluated in the liver as content as biomarkers of health status. Liver samples were collected to assess the expression of melatonin receptor and synthesis genes (*mel1b*, *asmt*), opsins (*tmt*, *opsin4*, *VA opsin*, *sws*), and stress-related genes (*sod1*, *sod2*, *hspa4b*). This study provides the evidence of opsins and melatonin-related hepatic gene expression in female swordfish, indicating a potential role of peripheral circadian rhythms in regulating physiological functions beyond reproduction. A significant modulation of *sws* expression was found between mature and immature females during the non-reproductive period. Health assessments revealed no major impairment, though mature females had a higher number and larger size melanomacrophage centers, likely linked to aging and prolonged exposure to environmental stressors. Increased numbers of melanomacrophages and upregulation of *hspa4b* in mature females suggest lingering stress effects from the reproductive season, though signs of recovery were observed compared to previous studies.



Animals, environment and microbiota: how microorganisms are changing the way we think about biodiversity conservation.

Giulio Galla¹, Lara Marinangeli^{1,2}, Lucia Zanovello¹, Barbara Crestanello¹, Matteo Girardi¹, Nadine Praeg³, Theresa Rzehak³, Julia Seeber^{4,5}, Paul Illmer³, Francesco Nonnis Marzano², Paolo Pedrini⁶, Giorgio Bertorelle⁷, Heidi C. Hauffe^{1,8}

¹ Conservation Genomics Research Unit, Centre for Research and Innovation, Fondazione Edmund Mach, San Michele All'Adige, Italy.

² Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma (PR), Italy.

³ Department of Microbiology, Universität Innsbruck, Innsbruck, Austria.

⁴ Institute for Alpine Environment, EURAC Research, Bozen, Italy.

⁵ Department of Ecology, Universität Innsbruck, Innsbruck, Austria.

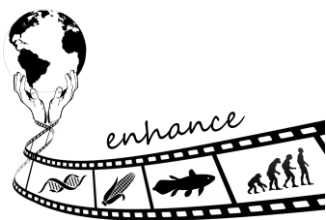
⁶ Research & Museum Collections Office, Conservation Biology Unit, Science Museum, Trento, Italy.

⁷ Population Genetics and Genomics Group, Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy.

⁸ National Biodiversity Future Centre (NBFC), Palermo, Italy.

Abstract

Diverse bacterial, fungal and viral communities characterize the intestinal flora and animal epidermis of all animal species and are known to provide essential services to host health. However, the role of this 'microbiota' in animal conservation has only recently been considered. Here we show how amplicon sequencing of the 16S rRNA gene and ITS2 locus from faecal samples and skin swabs collected non-invasively, allow us to study the diversity, composition and function of bacterial and fungal communities living in association with fauna, as well as to define the biological processes that characterize the network of interactions between species' communities and the surrounding environment ('ecosystem microbiota'). For example, alpine relictual populations of mountain hare (*L. timidus*) are threatened by climate and anthropic changes that result in snow cover reduction and habitat loss, as well as resource competition and potential hybridization with the European brown hare (*L. europaeus*). Using faecal pellets from an area of sympatry, we showed that the two species maintain distinct prokaryotic and fungal communities reflecting differences in host microbiota co-evolution. Furthermore, in both *Lepus* species, elevation influenced intraspecific gut fungal composition, but not prokaryotic diversity, highlighting differences in the resilience of these communities to environmental change. Interconnection between host-associated and environmental microbiota is further exemplified by the study of *Bombina variegata*. Here, the profiling the skin microbiota from individuals collected across different habitats highlighted a marked association between skin and water microbiota suggesting skin microbial diversity is affected by water quality parameters such as pH, dissolved oxygen, and temperature. The study of microbial diversity is proposed as an effective tool to estimate the effects of biotic and abiotic factors on the health of charismatic and/or endangered species, as well as changes in biodiversity through time.



Environmental contaminants and embryo development in *Caretta caretta* from two different sites of Mediterranean Sea

Erica Trotta¹, Paolo Cocci², Francesco Alessandro Palermo², Luana Papetti³, Giulia Chemello¹, Giorgia Gioacchini¹

¹ Department of Environmental and Life Science (DISVA), Polytechnic University of Marche, 60131, Ancona, Italy.

² School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III Da Varano, I-62032 Camerino, MC, Italy.

³ tartAmare, Centro di educazione ambientale e di didattica sul mare, Centro per la conservazione delle tartarughe marine, Via San Sebastiano 84, 58100 Grosseto, Italy.

Abstract

The Mediterranean Sea is increasingly subjected to anthropogenic pressures that threaten marine biodiversity and ecosystem integrity. Among the most affected species is the loggerhead sea turtle (*Caretta caretta*), the most widespread sea turtle in the region. It has been designated as a sentinel species and official bioindicator for Descriptor 10 “Marine Litter” under the European Marine Strategy Framework Directive. Loggerhead turtles are exposed to a wide range of environmental contaminants throughout their life cycle, including microplastics (MPs), heavy metals (HMs), persistent organic pollutants (POPs) and phthalates, which can bioaccumulate and impair key physiological functions. This study integrates morphological, physiological, histological, and toxicological approaches to investigate the presence and effects of pollutants, specifically MPs and polycyclic aromatic hydrocarbons (PAHs), in unhatched embryos collected from two Mediterranean nesting areas: the Ionian and Tyrrhenian Seas. Results revealed significantly higher rates of early developmental arrest and lower biometric parameters in Ionian embryos. MPs were detected in the liver tissues of 27 embryos from both nesting areas, with no significant differences between sites. However, a significant correlation was found between MPs abundance and the presence of hepatic melanomacrophages, a recognized biomarker of stress. PAHs were also detected in the liver and yolk of 7 embryos, with acenaphthylene being the most prevalent compound, along with protein levels of CYP1A1, a key enzyme involved in detoxification processes. These findings suggest not only the probable maternal transfer of contaminants via yolk deposition but also the active absorption and accumulation of pollutants in the developing embryo's liver. The ultimate goal of this approach is to develop a unified and easily applicable monitoring protocol that can be adopted across different Mediterranean regions. Such a standardized framework will enable more effective long-term assessment of environmental risks to *C. caretta*, supporting evidence-based conservation strategies and harmonized monitoring efforts at the regional scale.



Tracking population connectivity: mitogenome phylogeography of *Ixodes ricinus* across Europe and North Africa

Alessandro Nota¹, G. Lombardo², C. Croci¹, S. Melis¹, Juan Esteban Uribe², E. Pelloni¹, E. Moroni¹, E. Deagostino¹, D. Frigerio¹, D. Pistone¹, R. Di Gerlando^{1,3}, C. Fiorini⁴, L. Caporali⁴, V. Carelli⁴, M. Castelli¹, D. Sassera^{1,5}, A. Olivieri^{1,5}, C. Bandi^{2,6}, A. Torroni^{1,6}

¹ Department of Biology and Biotechnology L. Spallanzani, University of Pavia, Pavia, Italy.

² Department of Bioscience, University of Milan, Milan, Italy.

³ Molecular Biology and Transcriptomics Unit, IRCCS Mondino Foundation, Pavia, Italy.

⁴ IRCCS Institute of Neurological Sciences, Bologna, Italy.

⁵ NBFC, National Biodiversity Future Centre, Palermo, Italy. This project was funded under the National Recovery and Resilience Plan (PNRR), Mission 4 Component 2 Investment 1.4 – Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP F13C22000720007, Project title “National Biodiversity Future Center – NBFC”.

⁶ This project was financed by the Italian Ministry for Universities and Research (MUR) for the PRIN 2022 PNRR grant P2022JZKPY.

Abstract

Ixodes ricinus, the most widespread tick species in Europe, is a vector for many zoonotic pathogens that affect humans, domestic animals, and wildlife. The tick can feed on various vertebrates, thereby challenging prevention and conservation efforts and having a good potential for migration and gene flow. Understanding its phylogeographic structure is thus essential to shed light on its routes of dispersion and into dynamics of population expansion and contraction, helping also to forecast how environmental change may alter the distribution of this emerging threat.

Here, we sequenced 472 complete *I. ricinus* mitochondrial genomes from individuals collected in 68 locations across Europe and North Africa. Moreover, we added to our database 59 mitogenomes retrieved from public databases to further increase geographic coverage. We then reconstructed the phylogenetic history of the species using maximum parsimony, maximum likelihood, and Bayesian approaches.

Five major haplogroups emerged, with limited geographic clustering and notable gene flow across populations, likely driven by both mammal and bird migration. Haplogroup E corresponds to the congeneric *Ixodes inopinatus*, which one decade ago was proposed as a separate species. However, the limited divergence between this haplogroup and the others does not support its distinction as a different species, at least from a mitochondrial DNA perspective. Our data demonstrate the presence of this haplogroup in North Africa and in some Italian samples, consistent with bird-borne long-distance migration that could originate novel foci of tick-borne diseases.

By coupling a dense spatial sampling with high-resolution mitogenomics, our study identifies routes most likely to introduce tick-borne pathogens and offers a genomic baseline with which future range shifts can be detected. These findings will help to design targeted surveillance plans and mitigate the conservation impacts of vector-borne pathogens in a warming Europe.



2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

REPETITIVE DNA

Oral presentation,

Chairs: Eva Šatović Vukšić and Maria Assunta Biscotti



Genomic landscape of repetitive DNAs in Neotropical electric fishes

Camila do Nascimento Moreira¹⁻², William Gareth Richard Crampton³, Pedro Henrique de Andrade Affonso¹, Cesar Martins¹, Adauto Lima Cardoso¹

¹ Instituto de Biociencias, Campus de Botucatu, Universidade Estadual Paulista (UNESP), R. Prof. Dr. Antonio Celso Wagner Zanin, 250, Botucatu, SP 18618-689, Brazil.

² Centro de Estudos em Celulas Tronco, Terapia Celular e Genetica Toxicologica, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, 79080-190, Brazil.

³ Department of Biology, University of Central Florida, Orlando, FL, 32816, USA.

Abstract

The Neotropical electric fishes of the order Gymnotiformes, known for their unique electrogenic and electrosensory systems, provide exceptional models for investigating ecological and evolutionary questions. While their phylogenetic and cytogenetic diversity is well documented, information about the diversity and evolution of repetitive DNA in gymnotiform genomes remains limited. To understand how repetitive DNA has shaped genome evolution in this group, we conducted bioinformatic analyses on raw sequencing data and genome assemblies from multiple species representing three major families. Our analysis revealed that closely related species share similar patterns of repetitive DNA composition, with Gymnotidae and Aptereronotidae exhibiting the highest (16.5%) and lowest (9.4%) proportions of Repetitive DNA, respectively. We identified 40 satellite DNA families, with five exclusive to Gymnotidae, twenty-two to Hypopomidae, and six shared between Aptereronotidae species. Only one satellite DNA (NEFSat3-18) was conserved across all analyzed species, suggesting rapid evolutionary turnover of these sequences. The evolutionary dynamics of transposable elements varied among families, with Gymnotidae showing recent expansion of LINE elements and DNA transposons, while Aptereronotidae displayed more ancient patterns of transposon activity. Analysis of transposable element landscapes revealed that all species experienced at least one burst of transposition during their evolution. Our findings demonstrate that repetitive DNA diversification parallels the evolutionary history of gymnotiform species. We also present a comprehensive dataset of repetitive DNA sequences that can be used as cytogenomic markers in comparative and evolutionary studies.



Satellite DNA variation in species of the *Anopheles gambiae* complex provides new insights into mosquito evolution and vector biocontrol

Jiangtao Liang¹, and Igor V. Sharakhov¹

¹ Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Abstract

Heterochromatin, which is enriched in repetitive DNA sequences, plays a crucial role in genome stability, transcriptional regulation, and chromosome architecture. Investigating repetitive DNA enhances our understanding of heterochromatin evolution and function. The sibling species within the *Anopheles gambiae* complex, including major malaria vectors, provides a convenient system to study heterochromatin in malaria mosquitoes due to their recent evolution. To elucidate the chromosomal distribution of heterochromatin, we performed the first comprehensive analysis of male karyotypes from four sibling species in the *An. gambiae* complex: *An. coluzzii*, *An. arabiensis*, *An. quadriannulatus*, and *An. merus*. By integrating published data with our own, we confirmed previously reported repetitive sequences and identified multiple novel tandem repeats using Tandem Repeat Finder (TRF) and RepeatExplorer software. Our research focused on high copy number satellite DNAs. Based on monomer sequences identified by TRF and RepeatExplorer, we designed oligonucleotide probes and performed fluorescent in situ hybridization (FISH) on mitotic or meiotic chromosomes from all four species. Satellite DNA mapping revealed that high-copy tandem repeats are predominantly located in centromeric/pericentromeric regions of autosomes and heterochromatin regions of sex chromosomes, which undergo rapid turnover in the *An. gambiae* complex, although certain sequences remain conserved. Furthermore, based on the distribution patterns of repetitive DNA, we proposed a chromosomal phylogeny for the complex, which is partially consistent with previously published species phylogenies. Notably, the satellite DNAs identified on the sex chromosomes offer promising targets for advanced vector control strategies, such as CRISPR/Cas9-based X-chromosome shredders to manipulate the sex ratio of offspring.



Transposable elements triggered the genome diversification of Sigmodontinae rodents (Rodentia: Cricetidae)

Camila do Nascimento Moreira¹

¹ Saúde e Desenvolvimento da Região Centro-Oeste, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.

Abstract

The subfamily Sigmodontinae represents the largest group of Neotropical mammals, widely distributed from North to South America. These rodents present a highly chromosomal diversity that seems to be triggered by transposable elements (TE). Analysis of southern blotting and fluorescent *in situ* hybridization evidenced that basal tribes of Sigmodontinae, as Sigmodontini, retain LINE activity and present conserved karyotypes. On the other hand, derived tribes, such as Oryzomyini, where occurs the extinction of LINE, present highly rearranged karyotypes. In the absence of LINE, these genomes were colonized by another TE, the LTR/ERVs. Herein, the TE content in Sigmodontinae species was explored using *in silico* analysis in the genome assembly of three Sigmodontinae species, distributed across the phylogeny of the subfamily: *Sigmodon hispidus* as basal; *Phyllotis vaccarum* as intermediate; and *Holochilus nanus* as derived. TE was identified in the assemblies based on a custom repeat library created with RepeatModeler and annotated with RepeatMasker. Output files from RepeatMasker were used to plot a repeat landscape after the calculation of Kimura values. RepeatMasker analysis highlighted that 43,79 %, 44,00 %, and 36,16 % of the *S. hispidus*, *P. vaccarum*, and *H. nanus* assemblies, respectively, are composed of repetitive sequences. Repeat landscapes displayed waves of TE colonization in these genomes, *S. hispidus* presented a recent and huge wave of LINE, *P. vaccarum* presented a middle to new wave equally composed of LINE and LTR/ERVs, and *H. nanus* presented an older wave mainly composed of LINE and a newer predominantly composed of LTR/ERVs. These results reinforce the role of TE in the diversification of the Sigmodontinae subfamily. In addition, the expansion of LTR/ERVs in Oryzomyini, concomitant with the huge chromosomal radiation of the tribe, highlighted the tribe as a model to understand the impact of TE in the occurrence of chromosomal rearrangement in rodents.



Dispersed repeats in the *C. merolae* genome

Valentina Rudenko¹, Eugene Korotkov¹

¹ Institute of Bioengineering, Research Center of Biotechnology RAS.

Abstract

Repeated DNA sequences are widespread in eukaryotic genomes. They are located predominantly in heterochromatic and intergenic regions and, therefore, can be highly diverged. Most dispersed repeats (DRs) are mobile elements. Their nature determines the potential for adaptive and evolutionary changes in the genome. There are a large number of bioinformatics approaches to search for DRs. However, all of them allow detecting conservative repeats. The number of substitutions per nucleotide does not exceed 1. We developed the IP method to identify highly diverged DRs. The method consists of iterative procedure to create a repeat family profile, which is a positional-weight matrix. Then the profile is used in the dynamic programming algorithm to identify repeats belonging to the family. The search for DRs was carried out in the genome of the unicellular microalga *Cyanidioschyzon merolae*. It is believed that this microalgae can be grown in the sea water in coastal areas with high temperatures and is useful the field of bioproduction of proteins, oils, pigments. We found 20 DRs families which include 33,938 repeats in the *C. merolae* genome. DRs cover 72.6% of the genome; the number on the direct and reverse DNA strands in approximately the same. In the sequences of chloroplast and mitochondria, DRs were practically not found. The average length of repeats is 520 bp. We compared our results with the annotation of repeats in the *C. merolae* genome was carried out using popular computer programs RepeatMasker and RED. They were possible to detect only 20,320 DRs, which cover about 28% of the genome. These repeats are more conservative and they lengths mainly in the range of 100-300 bp. Thus, our study showed that the IP method reveals more diverged and longer DRs than existing approaches.



Independent Origins of Neo-Sex Chromosomes and Distinct Y Chromosome Degeneration Mechanisms: Satellite DNA vs. Transposable Elements in Two Membracidae Species

Pablo Mora¹, Ana E. Gasparotto¹, Ana B. S. M. Ferreti¹, José M. Rico-Porras², Teresa Palomeque², Pedro Lorite², Daniela Palmer Droguett³, Diogo C. Cabral-de-Mello¹

¹ Departamento de Biologia Geral e Aplicada, Instituto de Biociências/IB, UNESP—Universidade Estadual Paulista, Rio Claro, 13506-900 São Paulo, Brazil.

² Departamento de Biología Experimental, Área de Genética, Universidad de Jaén, Paraje las Lagunillas s/n, 23071 Jaén, Spain.

³ Biology Department, The University of Texas at Arlington, USA.

Abstract

The order Hemiptera, commonly known as true bugs, is an extensive group, ranked as the fifth-largest order of insects with almost 107,000 described species. This makes Hemipterans the largest order within the Hemimetabola. Among them, the suborder Auchenorrhyncha, specifically the family Membracidae, is notably diverse and morphologically varied. They are commonly referred as "true hoppers," and stands out for its exceptional mobility. These organisms possess a piercing-sucking feeding mechanism and are predominantly plant feeders (mainly monophagous), although some exhibit fungivorous behavior. Given their significant role as key vectors of plant pathogens and their importance as crucial ecological and evolutionary model systems, true hoppers play an integral role in both scientific research and society. Membracids, like other Hemipterans, exhibit holocentric chromosomes which are characterized by the lack of a single, localized centromere. The evolution of holocentricity has occurred independently at least 19 times in various clades across the tree of life. The presence of holocentric chromosomes has been hypothesized to impact an organism's propensity for chromosomal rearrangements, however the chromosomal evolution of Membracids remains poorly understood. Although, holocentric chromosomes are found across various clades they remain relatively enigmatic in terms of the genetic features and molecular mechanisms governing their fusion and fission. The modal chromosome number in membracids is $2n=21$, X0. However, in some species, the occurrence of the so-called neo-sex XY system has been described, as in *Calloconophora caliginosa* ($2n=18$, neoXY) and *Amblyophallus exaltatus* ($2n=20$, neoXY). In a comparative analysis of repetitive DNA sequences, we found that despite having "the same outcome" (neo-sex chromosomes), the differentiation process is completely different in both species, with *A. exaltatus* being governed by TEs and *C. caliginosa* by satDNA sequences.



2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

POSTERS

Conservation biology

Chairs: Federica Carducci and Giorgia Gioacchini



Genetic analysis for the conservation of secondary grasslands in central Italy

Chiara Spinsante¹, Marina Allegrezza², Marco Barucca¹, Maria Assunta Biscotti¹, Adriana Canapa¹, Elisa Carotti¹, Simona Casavecchia², Edith Tittarelli^{1,4}, David L. J. Vendrami³, Federica Carducci¹

¹ Department of Life and Environmental Sciences (Polytechnic University of Marche).

² Department of Agricultural, Food and Environmental Sciences (Polytechnic University of Marche).

³ Department of Department of Animal Behavior (University of Bielefeld).

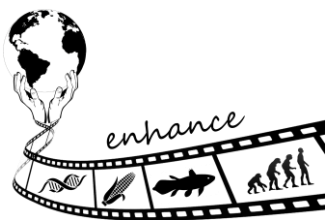
⁴ IUSS – School for Advanced Studies, Pavia.

Abstract

Secondary grasslands are semi-natural ecosystems resulting from the human transformation of forested environments, and they play a key role in biodiversity conservation where the presence of grazing animals promotes seed dispersal, facilitating the establishment of new plant species. The loss of these ecological practices can lead to a reduction in biodiversity and to the gradual colonization by shrub and tree species, transforming the grasslands into secondary woodlands.

In order to counteract biodiversity loss and allow the conservation of these ecosystems, the main goal of this work was to improve the quality of germplasm to be used in the formulation of seed mixtures intended for the seeding and overseeding of these secondary grasslands, offering a more effective alternative to the currently available commercial products.

In this study, we analysed a total of 45 individuals each of *Bromus erectus* and *Phleum ambiguum*, two grass species distributed across central Italy, sampled from three sites (Vallemontagnana, Monte Murano, and Castelletta), using double-digest Restriction-site Associated DNA sequencing (ddRAD). The results obtained for *B. erectus* showed a distinct genetic structure between the three different locations and the presence of kinship among some individuals, while *P. ambiguum* appeared genetically homogeneous across the three sampling sites, without any relationship between individuals. These results, together with the results obtained from genetic diversity and inbreeding analyses suggest the necessity to adopt differentiated strategies in seed selection for grassland restoration, in order to permit biodiversity conservation and ecosystem resilience.



***Prdx4* downregulation as a protective lipid-mediated response to PFAS exposure in *Squalius cephalus* kidney**

Sara Pacchini¹, Giacomo Vanzan¹, Elisabetta Piva¹, Laura Drago¹, Shaghayegh Kholdihaghighi^{1,2}, Martina Cortese¹, Paola Irato¹, Andrea Bottacin-Busolin³, Andrea Marion³, Gianfranco Santovito¹.

¹ Department of Biology, University of Padova, Padova, Italy.

² Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Venice, Italy.

³ Department of Industrial Engineering, University of Padova, Padova, Italy.

Abstract

Perfluoroalkyl substances (PFAS) are widely detected in the environment due to their persistence and mobility, raising concerns about their impact on freshwater organisms and ecosystems. PFAS exposure can induce oxidative stress either by disrupting antioxidant enzymes or by promoting reactive oxygen species (ROS) formation. Excess ROS may oxidise cellular macromolecules such as lipids and proteins, unless efficiently neutralised by the antioxidant system.

In this study, we investigated the physiological effects of chronic environmental exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), the two most toxic PFAS, in *Squalius cephalus*, a freshwater fish native to the Veneto region (Italy). We focused on the oxidative stress risk responses of the kidney, a primary organ for PFAS accumulation and excretion, sampled from 3 differently polluted sites.

Results revealed that these substances, even at a minimal level of contamination, can cause cellular damage through the oxidation of proteins, which are the major target of PFAS toxicity. However, in all sites, fish can still respond to the risk of oxidative stress, activating the antioxidant defences and preventing the damage to lipids, which remain unaltered even at high PFAS concentrations.

We examined gene expression of peroxiredoxin 4 (*Prdx4*), an antioxidant enzyme localised in the endoplasmic reticulum with two key functions: peroxide scavenging and inhibition of intracellular lipid accumulation to prevent steatosis. *Prdx4* mRNA was progressively down-regulated with increasing PFAS exposure, indicating a limited antioxidant role under these conditions. Instead, its down-regulation appears to contribute to enhancing lipid accumulation in the cytoplasm of renal cells. This cell vacuolisation may create a hydrophobic environment that limits PFAS bioaccumulation and reduces their ability to bind cellular proteins, thereby minimising further molecular damage.

These findings highlight a potential compensatory mechanism in *S. cephalus*, where lipid remodelling through *Prdx4* downregulation may help mitigate PFAS toxicity by preserving protein integrity.



The low-density lipoprotein receptor gene family: trying to reconstruct the evolutionary history in Chordata.

Alessandro Orsini¹, Chiara Spinsante¹, Maria Assunta Biscotti¹, Adriana Canapa¹, Elisa Carotti¹, Edith Tittarelli^{1, 2}, Federica Carducci¹, Marco Barucca¹

¹ Department of Life and Environmental Sciences (Polytechnic University of Marche).

² IUSS – School for Advanced Studies, Pavia.

Abstract

Despite the considerable number of papers published concerning the Low Density Lipoprotein receptors (LDLRs) gene family on their key role in binding lipoproteins, information regarding the evolutionary history of this gene family is still scarce. To fill this gap, we focused on the 15 members belonging to LDLR superfamily: Very Low-Density Lipoprotein Receptors (VLDLR) or Lipoprotein Receptor 8 (LR8), LDLR, Sorting-Related receptor with A-type repeats (SORLA), and 12 LDL Receptor-related Proteins (LRPs). Starting from a research conducted in our laboratory, we moved backward to the base of the vertebrate evolutionary lineage and beyond, taking into account *Polypterus senegalus*, *Callorhinchus milii*, *Petromyzon marinus*, *Myxine glutinosa*, and *Branchiostoma lanceolatum*. A total of 169 sequences were examined through Maximum Likelihood phylogenetic inference. In Cephalochordates, only one gene per group is present, suggesting that a second gene for each group emerged with Chondrichthyes, and a third gene (in the VLDLR–LDLR–LRP8 and LRP3–LRP10–LRP12 triads) appeared in Osteichthyes. Within each of the 15 clades, Sarcopterygii and Actinopterygii typically form two distinct monophyletic groups, except for LRP2, LRP4, and LRP11. In this latter, sequence of *C. milii* groups unexpectedly with *X. tropicalis*. In general, *C. milii* genes are placed outside the Sarcopterygii and Actinopterygii clusters. The study also confirms known gene duplications in Actinopterygii, especially in *T. rubripes*, which shares some duplications (like LRP2) with *L. oculatus* and *P. senegalus*. However, LRP4 and LRP6 are missing in *senegalus*, likely due to species-specific gene loss. Therefore, during the transition from Agnathans to Chondrichthyes, only 8 of the 12 genes expected from the 2R whole genome duplication were present, which could hint at their importance in shaping later gene families. Interestingly, the emergence of extra copies in the VLDLR–LDLR–LRP8 and LRP3–LRP10–LRP12 triads cannot be attributed to genome duplications.



Structure genetic analysis of the genus *Chamelea* in the whole mediterranean basin and beyond

Francesca Costante¹, Maria Assunta Biscotti¹, Adriana Canapa¹, Federica Carducci¹, Elisa Carotti¹, Chiara Spinsante¹, Edith Tittarelli^{1,3}, David L. J. Vendrami², Marco Barucca¹

¹ Department of Life and Environmental Sciences (Polytechnic University of Marche).

² Department of Department of Animal Behavior (University of Bielefeld).

³ IUSS – School for Advanced Studied, Pavia.

Abstract

Chamelea gallina is a bivalves mollusk belonging to the Veneridae family, commonly found in sandy coastal ecosystems. It lives at depths of up to 20 meters and it is one of the most important European fishery resource. However, overfishing combined to fluctuations of environmental and biological factors have led to a significant decline in this population in the last decades, threatening the biological and economic sustainability of this species. In line with these premises, it is mandatory to unravel the current state of population genetic structure to evaluate its genetic variability, an indicator of a population's health status.

To this aim, 160 specimens of *C. gallina* were considered, collected from eight sites in the Mediterranean Sea (Spain, Portugal, Sicily, Turkey and 4 sites on the Adriatic coast), together with 40 individuals of its congeneric *C. striatula* sampled from two sites in the Atlantic Ocean (Portugal and Dogger Bank).

Therefore, we used the double digest Restriction-site Associated DNA sequencing (ddRAD) technique, obtaining thousands of genetic markers (SNPs), which provide valuable insights into the population genetic structure of the genus *Chamelea*.

The results highlighted the presence of two distinct groups: one for *C. gallina* and the other for *C. striatula*. Further analysis on *C. gallina* showed a genetically distinct population from Turkey. More in detail, a separation was observed between individuals from Spain and Portugal, while those from Sicily were distributed across the Mediterranean basin, including the Adriatic Sea.

Focusing on the Adriatic coast, no significant genetic structure was detected among the samples from the four locations analysed, confirming our previous results in which all individuals belong to a single panmictic population.

The overall results will allow us to establish the current levels of genetic diversity for these species in order to determine if they are able to cope environmental changes.



Let it Bee, defending native *Apis mellifera ligustica* from hybridization

Elisa Carotti¹, Federica Carducci¹, Edith Tittarelli^{1,2}, Chiara Spinsante¹, David L. J. Vendrami³, Maria Assunta Biscotti¹, Marco Barucca¹, Adriana Canapa¹

¹ Department of Life and Environmental Sciences, Marche Polytechnic University.

² Scuola Universitaria Superiore Pavia - IUSS, Italy.

³ Bielefeld University, Germany.

Abstract

The European honey bee (*Apis mellifera*) is a key pollinator and an economically viable species managed for honey, wax and pollination. Its diversification has resulted in 31 subspecies grouped into five evolutionary lineages in different regions. Human practices like queen trade and migratory beekeeping have disrupted natural honeybee populations, leading to genetic mixing that threatens local diversity. Combined with climate change and disease, this contributes to global honeybee decline. Preserving native genotypes is crucial, as they are often better adapted and more resilient. Genomic tools, such as whole-genome sequencing (WGS), have become essential to monitor introgression and support breeding and conservation programs. In this study, genetic introgression was assessed in three *A. mellifera ligustica* queens in Parco del Conero, Italy, through WGS of 15 drones per apiary. The subspecies of *Apis mellifera* were used as reference to better analyse the three sites of interest and the results were compared with those of other reference subspecies, including *A. m. carnica*, *A. m. ligustica*, *A. m. mellifera*, *A. m. sicula* and the Buckfast bee. This latter bee, is a highly productive and disease-resistant hybrid, developed in England and widely used in European beekeeping, but it negatively impacts native bee populations. Genetic results indicate that the three Conero samples clustered with the *ligustica* subspecies even though there was some genetic variability within them. Globally, the results provide valuable insights for conservation strategies to preserve the genetic integrity of local honey bee populations.



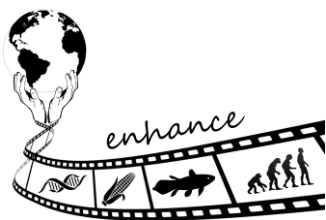
2nd Symposium
Environmental **cHANGes**,
Conservation biology,
and Evolution
ENHANCE

25th -26th June 2025, virtual

POSTERS

Repetitive DNA

Chairs: Federica Carducci and Elisa Carotti



Genome dynamics in Brachycera (Diptera): Evidence from heterochromatin organization, sex chromosome variation and rDNA localization

Rhavenna Thais Alves-Gomes¹, Ana Beatriz S. M. Ferretti¹, Gustavo H. M. Vazquez ², Pablo Mora¹⁻³; José Manuel Rico-Porras³; Cláudio J. von Zuben², Teresa Palomeque³; Pedro Lorite³; Patricia P. P. Maltempi¹, Diogo C. Cabral-de-Mello¹

¹ Department of General and Applied Biology, Institute of Biosciences, Sao Paulo State University (UNESP), Rio Claro, SP-Brazil.

² Department of Biodiversity, Institute of Biosciences, Sao Paulo State University (UNESP), Rio Claro, SP-Brazil.

³ Department of Experimental Biology, Genetics Area, University of J  n, J  n, Spain.

Abstract

Species of the suborder Brachycera (Diptera) hold significant economic, forensic, sanitary, and veterinary importances. Despite their relevance, cytogenetic and genomic information remains limited. To advance our understanding of chromosome organization and evolution in Brachycera, we studied the heterochromatin and 18S ribosomal DNA (rDNA) organization in *Chrysomya megacephala*, *Calliphora vomitoria*, *Calliphora vicina*, *Fannia pusio*, *Hermetia illucens*, *Lucilia cuprina*, *Lucilia sericata*, *Megaselia scalaris*, *Musca domestica*, *Ornidia obesa*, *Peckia australis*, *Peckia collusor*, and *Synthesiomyia nudiseta*. Most species exhibited the typical Brachycera karyotype of $2n = 12$, XY (male) and XX (female). We observed chromosomal variation in *F. pusio* ($2n = 12$, XY male/ $2n = 11$, XO female), *M. domestica* ($2n = 12$, XX female/ $2n = 11$, XO male), and *M. scalaris* ($2n = 6$ with homomorphic sex chromosomes). In most species, heterochromatin was present in the pericentromeric regions of autosomes. The X chromosome in most species is almost completely heterochromatic, and the Y chromosome shows variable heterochromatic amount. While heterochromatin localized to centromeres of autosomal chromosomes is common in Diptera, the X chromosome, unlike in most animal species, showed extensive heterochromatin content, indicating variability in sex chromosomes and degeneration. The rDNA was commonly located on sex chromosomes, as reported in other Diptera. However, in *P. australis*, *P. collusor*, *M. domestica*, *M. scalaris* and *S. nudiseta* it was found on autosomes, while in *H. illucens* and *C. vomitoria*, it was located on both. Our data provide evidence that while karyotype organization is largely conserved in Brachycera, extensive reshuffling occurred for the sex chromosomes, involving the degeneration of the X element through accumulation of repetitive DNAs. Future steps include a detailed analysis of repetitive DNA elements to elucidate the potential roles of different classes in the chromosomal variability observed in this group.



Heterochromatin expansion and repetitive element bursts drive genome enlargement in the Noctuid moth *Acyclania tenebrosa*

Ana Elisa Gasparotto¹, Ana Beatriz S. M. Ferretti¹, Fernanda A.F. Soares², Artur Orsetti², Wellington R. Clarindo², Diogo C. Cabral-de-Mello¹

¹ Department of General and Applied Biology, Institute of Biosciences, Sao Paulo State University – UNESP, Rio Claro, SP, Brazil.

² Department of General Biology, Viçosa Federal University – UFV, Viçosa, MG, Brazil.

Abstract

Noctuidae is one of the largest families of Lepidoptera, comprising more than 11,700 species, including several agricultural pests that are responsible for significant economic losses. Despite their importance, basic genomic features remain poorly understood. Here we advance on the better characterization of the genomic architecture of *Acyclania tenebrosa* by association of genomic and cytogenetic tools with emphasis on repetitive DNA content. The species presented 2n=62, WZ (female)/ZZ (male). Analysis of pachytene cells reveals high amount of heterochromatin, with occurrence of dots distributed along the entire length of all chromosomes. Chromosome mapping of 18S rDNA by Fluorescence *in situ* hybridization (FISH) revealed three bivalents harboring this gene, all in terminal position. The genome size of the species was enormous, with 2.14 pg of DNA amount. Repetitive DNA analysis were in accordance with the large genome size and high heterochromatin content, being revealed high abundance of satDNAs (14.3%) and TEs (53.6%). FISH mapping showed distinct chromosomal organization for satDNAs, as spread signals decorating the entire length of all chromosomes and single located clusters, while for TEs signals were predominantly enriched in the heterochromatin blocks, although the euchromatic regions also exhibited dispersed signals. These all genomic features observed in *A. tenebrosa* is highly divergent to observed in other Lepidoptera, that in general has small genomes, low heterochromatin content, low amount of satDNAs, and one cluster of rDNA. Our data reveals a complex diversification of repetitive elements in *A. tenebrosa*, enhancing the understanding of Noctuidae genome architecture and evolution.



Satellite DNAs in the *Bombyx mori* and *B. mandarina* genomes: relationship with transposable elements and low strain differentiation

José M Rico-Porras^{1,2#}, Pablo Mora^{1,2#}, Teresa Palomeque¹, Pedro Lorite¹, Diogo C Cabral-de-Mello²

¹ Department of Experimental Biology, Genetics Area, University of Jaén, Paraje las Lagunillas s/n, 23071 Jaén, Spain.

² Departamento de Biologia Geral e Aplicada, UNESP – Univ Estadual Paulista, Instituto de Biociências/IB, Rio Claro, São Paulo 13506-900, Brazil.

Abstract

Despite the economic and scientific importance of *Bombyx mori*, its satellite DNA (satDNA) fraction remains poorly characterized. Here, we present the first comprehensive analysis of the *B. mori* satellitome, revealing its low abundance (~0.76%), A+T enrichment, high monomer size variability, and predominantly dispersed chromosomal organization. Comparative analysis with *B. mandarina*, the wild ancestor of *B. mori*, demonstrated high satDNA conservation in abundance and sequence composition, despite speciation and domestication events. Divergence landscapes indicate predominantly ancient amplifications with few recent homogenization events. SatDNA families exhibited low strain differentiation across five *B. mori* strains. The W chromosome was identified as a hotspot for satDNA accumulation, especially for one satDNA family, which showed female-specific amplification and higher sequence homogeneity, suggesting functional or structural roles in W chromosome evolution. In contrast, the Z chromosome displayed similar satDNA content to autosomes, with two families exclusive to this chromosome in both *Bombyx* species. Remarkably, some of *B. mori* satDNAs were found to be derived from transposable elements (TEs), supporting the hypothesis of TE-driven satDNA origin via multiple mechanisms, including tandemization of TE fragments. Our findings underscore the dynamic interplay between satDNAs and TEs in shaping genome architecture, the stability of the satellitome through domestication, and the sex chromosome-specific accumulation patterns. These insights lay the groundwork for future studies investigating the functional roles of satDNAs in genome regulation and chromatin dynamics in Lepidoptera.



Comparative Analysis of TE Distribution in Autosomes and Sex Chromosomes of Six Squamate Species

Claudia Palladinelli¹, Elisa Carotti¹, Edith Tittarelli^{1,2}, Federica Carducci¹, Adriana Canapa¹, Marco Barucca¹, Maria Assunta Biscotti¹

¹ Department of Life and Environmental Sciences, Polytechnic University of Marche.

² IUSS - School for Advanced Studies, Pavia.

Abstract

Transposable elements (TEs) play a pivotal role in the evolution of vertebrate genomes, influencing genome size, structure, and dynamics. In reptiles, and particularly in squamates (lizards and snakes), TEs show a remarkable diversity and activity suggesting a highly dynamic genomic environment in squamates. Moreover, TEs are implicated in the shaping of sex chromosomes. Reptiles are a group characterized by both genotypic (XY/ZW) and temperature-dependent sex determination systems. The accumulation of TEs, especially on the W and Y chromosomes, may contribute to their heterochromatic and degenerate state. Indeed, the scientific community proposed that TEs can influence sex-specific gene expression and may play a role in the evolution of reproductive strategies. In this study, we analyzed six squamate species: *Crotalus adamanteus*, *Anolis carolinensis*, *Cyclura pinguis*, *Lacerta agilis*, *Podarcis siculus*, and *Podarcis bocagei*. Among these, *Anolis carolinensis* and *Cyclura pinguis* possess an XY sex determination system, while the remaining species exhibit a ZW system. For each species, after constructing a species-specific TE library through a *de-novo* bioinformatic pipeline, we assessed the contribution of TEs to both autosomal and sex chromosomes. Additionally, we evaluated the age of TE insertions through Kimura landscape analysis. Investigating the TEs contribution in reptiles this study can shed light on squamate genome evolution, sex chromosome differentiation, and the evolutionary forces shaping genomic architecture.



Comparing transcriptional activity of transposable elements between *Xenopus tropicalis* and *Xenopus laevis* during embryonic development

Natasha Albanese¹, Elisa Carotti¹, Federica Carducci¹, Edith Tittarelli^{1,2}, Chiara Spinsante¹, Adriana Canapa¹, Marco Barucca¹, Maria Assunta Biscotti¹

¹ Department of Life and Environmental Sciences, Polytechnic University of Marche, Italy.

² Scuola Universitaria Superiore Pavia - IUSS, Italy.

Abstract

Transposable elements are mobile DNA sequences that contribute to genome plasticity, gene regulation, and genome reorganization. During embryonic development, their activity is often tightly regulated and stage specific. The genus *Xenopus* represents an ideal model system to study these phenomena. In particular, *Xenopus tropicalis* (diploid) and *Xenopus laevis* (allotetraploid) offer a unique opportunity to compare TE activity in two distinct genomic contexts.

The aim of this study is to perform a comparative analysis of the transcriptional and temporal distribution of TEs during embryonic development in *X. tropicalis* and *X. laevis*, with a focus on expression patterns across major TE classes (DNA transposons, LINEs, LTRs, non-LTRs, retroelements, SINEs, and others) throughout key developmental stages (zygote, blastula, gastrula, neurula, tailbud, tadpole).

In *X. laevis*, TE expression ranges from 9.5% at the zygote stage to 13.5% in the tadpole, with clear peaks at the blastula and tadpole stages. DNA transposons are predominant across all stages, followed by LINEs and LTRs. In contrast, *X. tropicalis* exhibits a more pronounced TE activity, exceeding 25% at the blastula and tailbud stages, and displays a more diverse composition including LINEs, LTRs, and non-LTRs.

Temporally, Kimura divergence analysis reveals recent transpositional activity in *X. tropicalis*, while *X. laevis* shows a more complex temporal stratification, with both ancient and recent insertions distributed across its L and S subgenomes.

In conclusion, *X. tropicalis* displays an intense and coordinated transpositional activation, likely facilitated by its diploid genome structure, whereas *X. laevis* exhibits a more conservative and stratified expression pattern, reflecting its allotetraploid evolutionary history. These differences suggest a functional and species-specific role of TEs during embryonic development in the two *Xenopus* species.



Unlocking the Secrets of Thermal Stress: Transposable Elements in *Trematomus bernacchii*

Edith Tittarelli^{1,2}, Elisa Carotti¹, Claudia Palladinelli¹, Chiara Spinsante¹, Federica Carducci¹, Adriana Canapa¹, Marco Barucca¹, Maria Assunta Biscotti¹

¹ Department of Life and Environmental Sciences, Polytechnic University of Marche.

² IUSS - School for Advanced Studies, Pavia.

Abstract

Transposable elements (TEs) are mobile DNA sequences that play a crucial role in genome regulation, structure and evolution. Their activity is regulated by the host silencing mechanisms, such as DNA methylation, histone modifications and RNA interference, which prevent negative effect due to their mobilization. However, environmental stressors have the potential to destabilize these regulatory systems, leading to increased TE transposition. An increasing number of papers has emphasized the dual nature of TEs: while they can be viewed as genomic parasites, they also represent a significant source of genetic variability, facilitating rapid adaptation to changing environments. The TEs transcriptional responses to abiotic changes in fish are well documented in the scientific community. This study aims to investigate this phenomenon in *Trematomus bernacchii*, an Antarctic species. *T. bernacchii* is considered a model organism to study the molecular responses to heat stress. In light of the ongoing challenges posed by climate change, the aim of this study is to explore how *T. bernacchii* copes with thermal stress at the molecular level. Specifically, we analyzed TE transcriptional activity in two tissues, liver and gills, of specimens exposed to elevated temperatures (+1°C and +3°C) for periods of 5 and 15 days, compared to control specimens. Our results indicate that TE transcriptional activity is significantly affected by heat stress. We also evaluated the transcriptional activity of genes involved in TE silencing mechanisms. The expression of genes from the Argonaute family, those associated with heterochromatin formation, and components of the NuRD complex, active participants in TE repression, demonstrated distinct patterns depending on temperature conditions and tissue type. In conclusion, these findings suggest a tissue-specific, complex response to thermal stress in *T. bernacchii*, with TEs and their regulatory systems dynamically reacting, indicating the early stages of adaptive stress responses in this cold-adapted species.