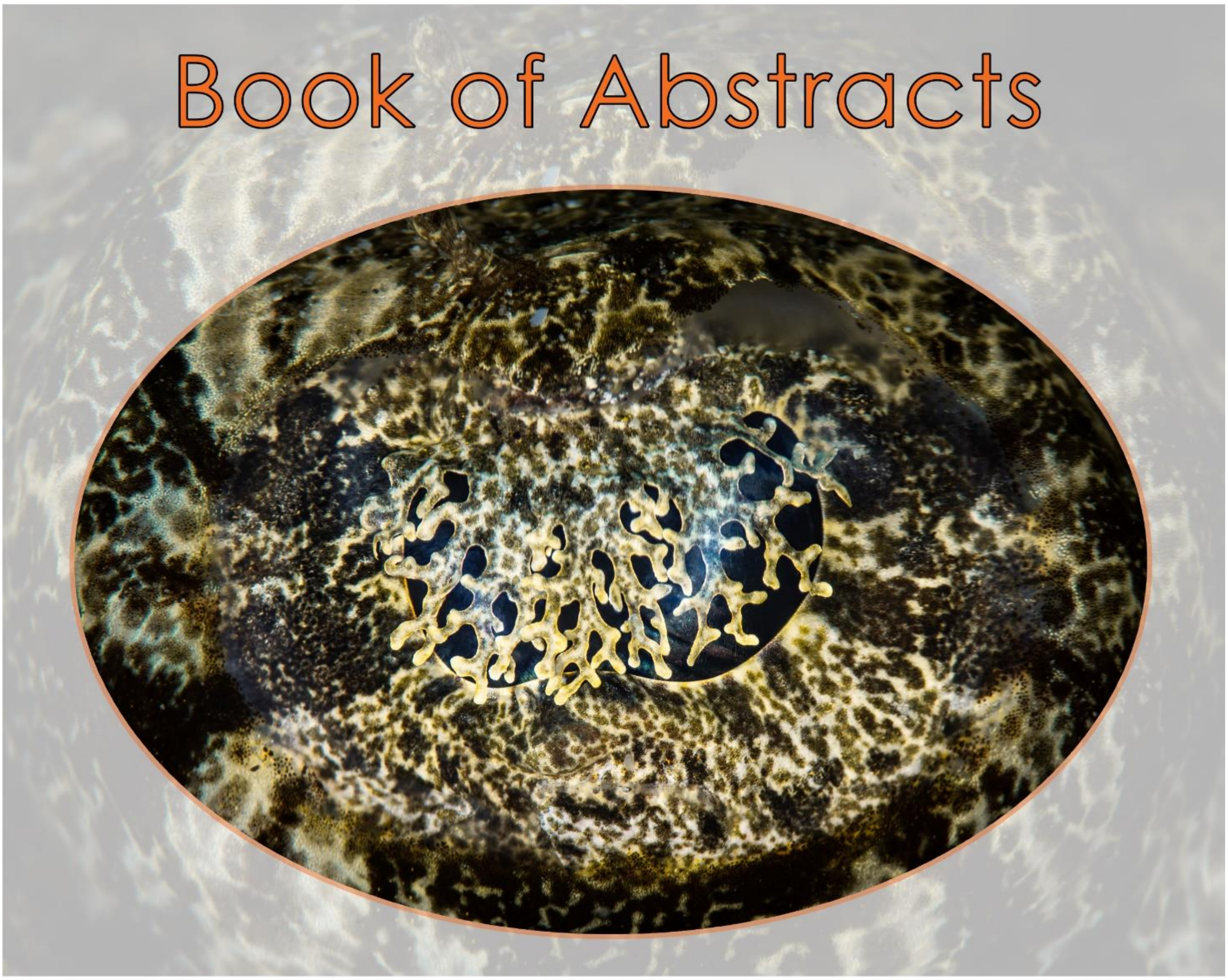
2nd Congress on MARINE EVOLUTION

EVOLMAR 2023

r-Evolutionary routes in the Sea

14-17 November 2023
www.evolmar.it

Book of Abstracts

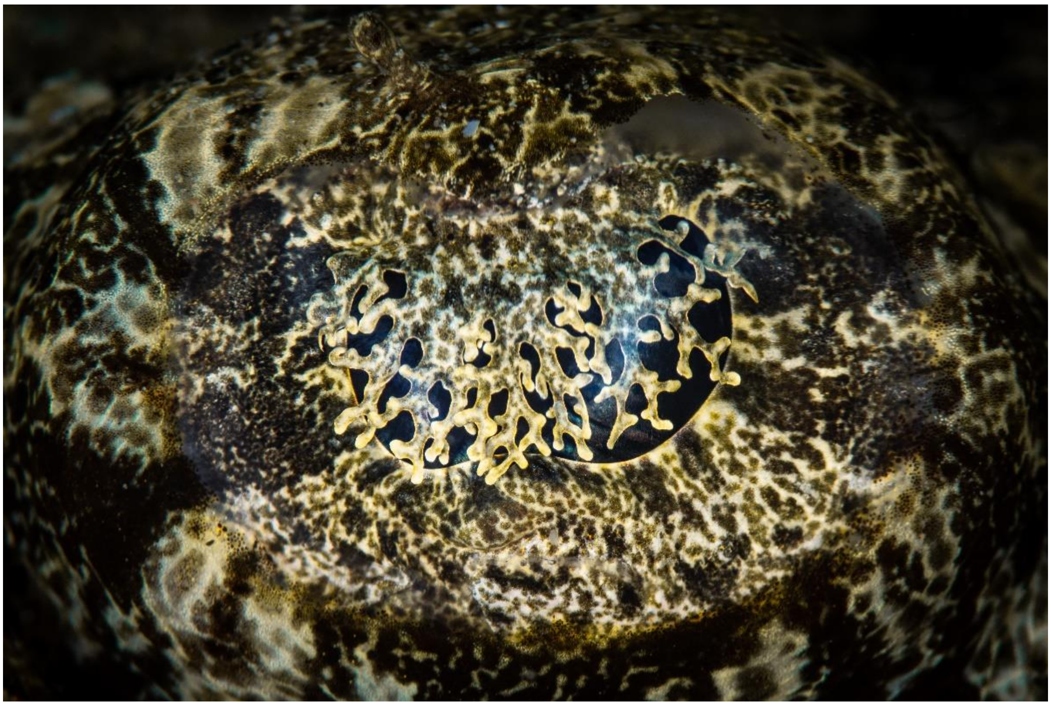


EVOL
MAR

SIBE
SOCIETÀ ITALIANA DI BIOLOGIA EVOLUZIONISTICA

Book of Abstracts

14–17 November 2023



SIBE
SOCIETÀ ITALIANA DI BIOLOGIA EVOLUZIONISTICA

Patronage

F I COMITATO SCIENTIFICO PER LA FAUNA D'ITALIA

NATIONAL BIODIVERSITY FUTURE CENTER

STAZIONE ZOOLOGICA ANTON DOHRN · SZN

UNIVERSITÀ DEGLI STUDI DI PADOVA

Sponsors

CARMAR ITALIA

DeBio DEPARTMENT OF BIOLOGY

frontiers

ISOTTA www.isotecnic.it

Physalia Courses

Scubalandia 潜水地

SIBE SOCIETÀ ITALIANA DI BIOLOGIA EVOLUZIONISTICA

UNIVERSITÀ DEL SALENTO

MTB Tourism and Biodiversity

Congress & Event Organization
M.T.B. Management of Tourism and Biodiversity
Elly Travel srl
Via al Quarto Miglio, 63 - 00178 Roma
Mobile +39 3491344831
cristina.compagno@ellytravel.com

Organizing Committee

Chair: **Giulia Furfaro**
Università del Salento, Lecce (Italy)

Lisa Locatello
Stazione Zoologica Anton Dohrn, Naples (Italy)

Marco Munari
Università di Padova, Padova (Italy)

Giulia Fassio
Sapienza Università di Roma, Roma (Italy)

Daniela Pica
Stazione Zoologica Anton Dohrn, Calabrian Marine Centre, Amendolara (Italy)

Donato Giovannelli
Università Federico II, Napoli (Italy)

Cristina Compagno
Congress Secretariat, M.T.B. Management of Tourism and Biodiversity

Michele Solca
Museo di Biologia Marina Pietro Parenzan, Lecce (Italy)

Web Design: **Giuseppe d'Errico**

Logo: **Daniela Ronchetti**

Photo Credits: **Michele Solca**

Cover Photo Credits: **Yann Toso** (photo title: *Mimic Eye*)

Scientific Committee

Chair: **Maria Vittoria Modica**
Stazione Zoologica Anton Dohrn, Naples, Italy

Lucia Bongiorni
CNR Consiglio Nazionale delle Ricerche, ISMAR Istituto di Scienze Marine, Venice, Italy

Emanuela Dattolo
Stazione Zoologica Anton Dohrn, Naples, Italy

Francesca Leasi
University of Tennessee at Chattanooga, USA

Luca Mirimin
Atlantic Technological University, Galway, Ireland

Luigi Musco
University of Salento, Lecce, Italy

Marco Passamonti
University of Bologna, Italy

Paolo Sordino
Stazione Zoologica Anton Dohrn, Naples, Italy

Sergio Stefanni
Stazione Zoologica Anton Dohrn, Naples, Italy

Emiliano Trucchi
Marche Polytechnic University, Ancona, Italy

EVOLMAR 2023

r-Evolutionary routes in the Sea

The Congress on Marine Evolution, EVOLMAR 2023 *r-Evolutionary* routes in the Sea took place in a virtual format on November 14th-17th, 2023. The Congress was co-organized by the Italian Society for Evolutionary Biology (SIBE-ISEB) and supported by prestigious Patronages and Sponsors. The meeting assembled international experts in Marine Evolutionary Biology to present the latest findings and diverse perspectives on the multiple patterns and processes at all levels of biological organization, inspiring insights into emerging networks and *r-Evolutionary* trajectories. The meeting featured a combination of invited keynotes, contributed talks and posters, for a total of 92 presentations arranged in a program revolving around 5 thematic areas: adaptation, biodiversity, conservation, macroevolution, populations and species. All abstracts submitted are included in this book. Some of the abstracts were edited to conform to the meeting format. The abstract content was not modified and corresponds to what originally submitted by the authors. The name of the presenting author is underlined.

Keynote Speakers

Jeanne Serb - ADAPTATION
Department of Ecology, and Organismal Biology,
Iowa State University, Ames, IA, USA

Andrea Quattrini - BIODIVERSITY
Department of Invertebrate Zoology, National Museum of
Natural History, Smithsonian Institution, Washington, DC, USA

Toby Daly-Engel - CONSERVATION
Department of Ocean Engineering and Marine Sciences,
Florida Institute of Technology, Melbourne, FL, United States

Nicolas Puillandre - MACROEVOLUTION
Institut de Systématique, Évolution, Biodiversité (ISYEB),
Muséum National d'Histoire Naturelle, CNRS, Paris, France

Thorsten Reusch - POPULATION & SPECIES
GEOMAR Helmholtz-Centre for Ocean Research
Kiel and Kiel University, Kiel, Germany

Special Guest

Laurent Ballesta

Naturalist photographer, six times winner of the Wildlife Photographer of the Year, awarded by the National Museum of London, he is the first photographer, in the last forty years of the competition, to receive the Grand Title twice: in 2021 and 2023.

‘Gombessa: The Mystery of the Rings’

Andromède Océanologie - ballesta.laurent@gmail.com

At the north of Cap Corse, more than 100 meters under the sea, there are strange formations that have never been explained before: 1,417 perfectly circular rings, 20 meters in diameter. The Gombessa expedition team, led by biologist and photographer Laurent Ballesta, intends to resolve this mystery and discover its origin. In collaboration with 41 French and foreign scientists, Laurent Ballesta's team carried out a whole series of protocols aimed at understanding the origin and functioning of these rings: their age, their dynamics of change, but also to identify the associated biodiversity. Laurent will be presenting some of the results of this 4-years work.

Adaptation

Chairs: **Luigi Musco** & **Marco Passamonti**

This theme encompasses research on molecular, anatomical, behavioral, and physiological adaptations in the marine environment, including the effects of sexually selected traits.

Adaptation

The interplay of adaptation and exaptation in the eyes of pteriomorphian bivalves

Jeanne M. Serb[1]

[1] Department of Ecology, Evolutionary, and Organismal Biology, Iowa State University, Ames, USA.

Aim

Organisms detect environmental stimuli using an array of receptors, and the duplication and divergence of these receptors provide evolutionary opportunities for species expansion into new ecological niches. Pteriomorphian bivalves have a myriad of light sensing organs, including eyes, that have evolved multiple times across, and within, lineages. Recent work indicates that eyes may be the result of co-option: sharing and/or diversification of preexisting genes that were once used in other light-sensing tissues. Thus, understanding the origin of eyes can provide insight into how complex phenotypes can arise through redeployment and elaboration of genetic pathways.

Methods

To examine the origin of eye types in pteriomorphian bivalves, we examined gene family expansion of opsin, the protein component of photopigments. We compared genomic content across Mollusca and spatiotemporal gene expression across developmental stages of bivalve species. Using a heterologous gene expression system, we are testing light-sensitivity of these opsin proteins.

Results

After an analysis of 80 molluscan genome assemblies, we found extensive fluctuations in opsin number which appear to be limited to bivalves and gastropods. Surprisingly, eyeless bivalves have the highest numbers of opsins, indicating that neither the presence nor the complexity of eyes are tied to an increase in gene copy number. When comparing eyed lineages, we found that opsin content and expression patterns vary among eye types.

Main conclusion

At the genetic level, eyed lineages do not follow the same evolutionary trajectory to achieve photosensitivity. Furthermore, the extensive diversification of opsins in eyeless species may indicate some opsins are multisensory receptors with light-independent functions. This hypothesis will require functional assays of the protein and allow for direct testing of adaptation.

Adaptation

Time to leave Neverland: Decoding the timing mechanisms of maturation and reproduction in the Sea

Gabriele Andreatta[1], Birgit Poehn[1], Federico Scaramuzza[1], Alexander Stockinger[1], Aida Coric[1], Lukas Orel[1], Florian Raible[1], Kristin Tessmar-Raible[1,2,3]

[1] Max Perutz Laboratories, University of Vienna, Vienna, Austria.
[2] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany.
[3] Carl-von-Ossietzky University, Oldenburg, Germany.

Abstract

Animals exploit environmental cues to orchestrate key events in their life-cycles. However, this fundamental synchronization is challenged by climate change and anthropogenic impact, making the understanding of the underlying mechanisms paramount for species and ecosystems survival. We use the genetically-tractable marine bristleworm *Platynereis dumerilii* to investigate how sexual maturation and reproduction are properly timed. This species coordinates such processes with the lunar cycle, a very widespread phenomenon in marine environments, yet poorly understood at the molecular mechanistic level. We show that a simple loss-of-function mutation in the *light-receptive cryptochrome* (*lcry*) gene, sufficient to alter the worm´s lunar reproductive rhythms in lab conditions, significantly impacts a variety of life-history traits. Indeed, knockouts display dampened rate of body and germline growth, delayed sexual maturation, which expands worms´ overall lifespan, as well as increased adult size and fecundity. Quantitative transcriptomics performed in different developmental stages identifies the endocrine system as significantly de-regulated in *lcry* knockouts. In particular, our analyses suggest that annelid sexual development and growth are coordinated by a combination of hormonal players regulating such aspects in either arthropods or vertebrates. In sum, our study reveals remarkable changes in life-history traits associated to the loss of a photoreceptor, identifies novel molecular mechanisms synchronizing animal reproduction with the lunar cycle, and unveils unexpected similarities in the endocrine dynamics underlying invertebrate and vertebrate sexual maturation. These aspects result particularly relevant to better understand the consequences of light pollution in the marine ecosystem, and shed light on the evolution of the molecular machinery which orchestrates animal reproduction.

Adaptation

Impact of the ejaculate microbiome on the outcome of sperm competition

Antonia Bruno[1], Anna Sandionigi[2], Lisa Locatello[3]

[1] Department of Biotechnologies and Biosciences, University of Milano-Bicocca, Milan, Italy.
[2] Quantia Consulting Srl, Milan, Italy.
[3] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Fano Marine Center, Fano (PU), Italy.

Aim

Despite the growing interest in the impact of microbial communities on host ecology, evolution, and fitness, very little is known on the reproductive microbiome. Microbes can affect sperm performance and fertilising efficiency and this effect is expected to be particularly pronounced in polyandrous species, where any reduction in fertilising efficiency can be exploited by rival ejaculates.

In this pioneering study, we delve into the largely uncharted domain of ejaculate microbiomes in model fish exhibiting guard-sneaker male mating tactics, the grass goby *Zosterisessor ophiocephalus*. The grass goby gave the first evidence of tactic-related mechanisms of either rival ejaculate exploitation or impairment mediated by seminal fluid. Considering the impact of microbes on sperm performance observed in other vertebrates, we expect a role of the microbiome of seminal fluid in the outcome of competing interaction between rival ejaculates observed in the grass goby.

Methods

We set up a method to extract bacterial DNA from the viscous seminal fluid, obtained by male seminal vesicles (i.e., accessory structures to the testes) during the reproductive season. High-throughput DNA sequencing allowed us to define the microbial composition and community structure in the seminal fluid of the two male phenotypes.

Results

Our results revealed a highly biodiverse composition, despite the low biomass, with 176 families from 22 different phyla. α- and β-diversity metrics revealed a significant difference in microbial composition between territorial and sneaker males. Notably, a bacterium belonging to the family *Mycoplasmataceae* showed a significant difference in relative abundance between the two groups, being a promising candidate for further *in vitro* experiments.

Main conclusion

Our preliminary findings suggest a potential role of the ejaculate microbiome in the competition for fertilisation. Future studies are warranted to uncover the mechanisms underlying these observed differences and to elucidate the potential adaptive benefits associated with these unique microbiomes.

Adaptation

The balance between transposable elements and their silencing mechanisms: environmental adaptation of ray-finned fish

Elisa Carotti[1], Federica Carducci[1], Marco Barucca[1], Adriana Canapa[1], Maria Assunta Biscotti[1]

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

Aim

Transposable elements constitute one of the main components of eukaryotic genomes. Due to their transposition ability, TEs can also be responsible for genome instability, and thus silencing mechanisms were evolved. However, TEs are not merely silenced rather, they can be domesticated for the regulation of host-coding gene expression, permitting species adaptation and resilience.

Therefore, the aim of this work was to investigate the impact of TEs in genome composition and how their activity and related silencing mechanisms respond to variation of abiotic factors in teleosts. Ray-finned fish are adapted to different ecological niches and are responsive to environmental changes.

Methods

Bioinformatic analyses were performed to investigate the transcriptional activity of TEs and genes involved in silencing mechanisms.

Results

We investigated the transcriptional contribution of TEs in the gill transcriptomes of three fish species exposed to different salinity conditions. We considered *Anguilla marmorata* and *Oncorhynchus keta*, both diadromous, and *Oryzias melastigma*, an euryhaline organism *sensu stricto*. Our analyses revealed an interesting activity of TEs in the case of juvenile eels, commonly adapted to salty water, when exposed to brackish and freshwater conditions.

Moreover, an activity of TEs was recorded also in the liver of the stenothermal fish *Puntius tetrazona*, after exposure at 13 °C indicating this tissue as target organ for this kind of stress.

Our results evidenced for the first time the activity of NuRD complex, a TE silencing mechanism typical of sarcopterygians, also in actinopterygians. For its functioning, we proposed an interaction between a krüppel-associated box (KRAB)-like protein specific to actinopterygians and TRIM33 tested by structural prediction and CoIP analyses.

Main conclusion

Therefore, our results highlight the importance of exploiting silencing mechanisms for TE control to rewire gene expression networks allowing species adaptation and resilience, challenging the general view of TEs as threatening elements.

Adaptation

Physiological and behavioral responses of the sea urchin *Arbacia lixula* from volcanic CO_2 vents

Ilaria D'Aniello[1], Maria Nardiello[1], Simonetta Fraschetti[1], Katiuscia Petrosillo[2], Folco Giomi[3], Antonino Miccio[4], Maria Gabriella Marin[5], Marco Munari[5]

[1] Department of Biology, University of Naples Federico II, Italy.
[2] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy.
[3] Independent researcher.
[4] Marine Protected Area 'Regno di Nettuno'.
[5] Department of Biology, University of Padova, Italy.

Aim

The increase of CO_2 into the atmosphere, as a result of human activities, is mitigated by ocean's absorption processes, which lead to a gradual decrease of ocean pH. This phenomenon, known as Ocean Acidification (OA), has a significant impact on the biology of various marine species, particularly those that build calcium-based structures. However, some species seem more resistant than others in copying with acidic conditions, showing a high adaptation potential. In this context, it is fundamental to understand what are the biological mechanisms determining this resistance. This study focused on exploring how chronic and acute exposure to acidic conditions affects the physiology and behavior of the sea urchin *Arbacia lixula*, studying populations which resides near the natural CO_2 vents in Ischia, Italy.

Methods

Specimens of adult sea urchins were sampled from two distinct populations along the pH gradient generated by the vents: S1 (mean pH 8.1; representing the ambient area) and S2 (mean pH 7.7; representing acidified area). The experimental setup involved exposures of animals from both S1 and S2 populations to both tested pH, using seawater collected on-site. Physiological aspects such as respiration and excretion, along with behavioral traits including righting and sheltering time, were assessed. All trials were replicated four times during the year.

Results

The findings reveal a persistent impact of the area on the respiration rate of mature sea urchins, specifically observed during the second and third sampling periods. The excretion rate was influenced by pH conditions only in October. Both pH and area factors influenced behavioral aspects across all temporal replicates, with a significant effect of their interaction on righting time.

Main conclusion

The variations in responses observed at different sampling times suggest the potential involvement of additional factors, beyond acidification, in the modification of the species' physiology and behavior. Nevertheless, our results offer initial insights into the direct effects of acidification on *A. lixula* within their natural habitat.

Adaptation

Assessing the resilience of the coralline macroalgae *Ellisolandia elongata* to high irradiances and desiccation in natural habitat

Rosa Donadio[1], Maria Luisa Pica[1], Ermenegilda Vitale[1], Giulia Costanzo[1], Alberto Colletti[1], Erika Fabrizzi[1], Simonetta Fraschetti[1,2,3], Carmen Arena[1,3]

[1] Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy.
[2] CoNISMa, Rome, Italy.
[3] NBFC-National Biodiversity Future Center, Italy.

Aim

Calcified algae are less common or absent in intertidal regions because they are particularly vulnerable to desiccation. Among corallinaceae, *Ellisolandia elongata* (J. Ellis & Solander) inhabits intertidal rock pools where large fluctuations in physic-chemical variables occur daily and represent a suitable model to study the capability of this species to cope with environmental constraints. To pursue this aim, individuals of *E. elongata* were sampled in the Marine Protected Area of the Gaiola Underwater Park (Gulf of Naples, Italy) during a prolonged low tide event occurring in early February to assess the photosynthetic efficiency and the occurrence of stress-defense ecological strategies in both submerged and exposed thalli.

Methods

Photosynthetic-light curves (LRC) were performed in vivo in exposed semi-bleached and submerged thalli utilizing a pulse amplitude-modulated fluorometer (Junior-PAM, Walz Gmbh, Effeltrich, Germany). On samples, the expression of the D1 protein of photosystem II, photosynthetic pigments, and antioxidant activity were also evaluated to study the adaptive response of *E. elongata* photosynthetic apparatus.

Results

At over-saturating irradiances, the prolonged low tide event determined, surprisingly, an increase of photosynthetic efficiency and a decline of non-photochemical thermal dissipation processes in exposed compared to submerged thalli together with a reduction of maximum photochemical efficiency, D1 protein expression, photosynthetic pigment content, and antioxidant activity.

Main conclusion

The prolonged exposure of submerged thalli to multiple stresses (i.e., high irradiance and desiccation) may have triggered a regulation strategy by which photosystem II is down-regulated to avoid photoinhibition of photosynthetic apparatus. The decreased antioxidant activity confirmed scavenger molecules' crucial role in lowering oxidative stress at the cellular level, contributing to photoprotection in *E. elongata*.

Adaptation

Whole genome duplication and gene evolution in the hyperdiverse venomous gastropods

Sarah Farhat[1], Maria Vittoria Modica[2], Nicolas Puillandre[1]

[1] Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France.
[2] Department of Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Roma, Italy.

Abstract

Venomous animals, such as snakes, spiders, or scorpions, represent a fascinating example of diversity in both taxonomy and function, found across at least 101 independent lineages. Despite their varied origins, these animals have evolved specialized structures for producing, storing, and delivering venom—a complex mixture of toxins primarily composed of proteins and peptides. These toxins serve diverse functions acting synergistically to incapacitate their target organisms. One group of diverse predatory marine organisms, the Neogastropoda, displays an impressive range of feeding strategies, including envenomation, asphyxia, and vampirism. However, little is known about the link between the diversity of these compounds and the hyperdiversification of neogastropod species, and how genome evolution is related to both the compounds and species diversities. Only eight neogastropod genomes have been sequenced, and there is uneven quality assembly among the 45 gastropod genomes sequenced so far. To address this gap, we have generated high-quality chromosome-level assemblies of two species: *Monoplex corrugatus* (tonnoidean) and *Stramonita haemastoma* (neogastropod). These genomes provide valuable references for their respective taxa and facilitate the identification of genome-level processes that contribute to the evolutionary success of predatory neogastropods. Through the analysis of syntenic blocks, Hox gene cluster duplication and synonymous substitutions distribution pattern, we inferred the occurrence of a whole genome duplication event in both genomes. As these species are known to release venom, toxins were annotated, but few of them were found in homologous chromosomes. Using transcriptomes data from two tissues in *S. haemastoma*, a comparison of the expression of ohnolog genes (orthologs found in homologous chromosomes in each species), where both copies were differentially expressed, showed that most of them had similar expression profiles. By conducting this study, we shed light on the relationship between genome evolution, the diversity of venom compounds, and the hyperdiversification of neogastropod species.

Adaptation

Biological adaptations to climate change and ocean acidification convey the need for considering their influences on toxicological testing in regulatory protocols: evidence from 20 years of biological tests with *Paracentrotus lividus*.

Andrea Gaion[1], Guido Scatena[1], Cristina Andra Vrinceanu[2], Davide Sartori[1]

[1] Italian Institute for Environmental Protection and Research (ISPRA). Via del Cedro 38, Livorno, Italy.
[2] Nottingham Geospatial Institute, University of Nottingham, NG7 2TU Nottingham, United Kingdom.

Aim

With specific reference to marine ecotoxicology, a crucial problem still to be resolved is understanding how a changing ecosystem could alter the existent toxicity of pollutants. Paracentrotus lividus has historically been used as model organism for scientific investigation, and to evaluate the toxicity of environmental matrices in Italian legislation. Our last published work (https://doi.org/10.1016/j.marpolbul.2023.115274) allowed us to demonstrate how the natural population of Livorno changed its sensitivity towards Cu under climate change conditions in the last 20 years.

Methods

We measured Cu EC_{50} values obtained over approximately two decades, using wild specimens of *P. lividus* and their correlations with environmental variables, calculated using satellite observations (Copernicus). Animals have always been collected in the same natural site, unaffected by important anthropogenic activities and exposed to the same transport, preparation, and testing protocols.

Results

From the correlation analysis conducted between EC_{50} and all the environmental variables considered, it can be inferred a stronger association relative the last 6 years compared to the other time interval. For all environmental variables, the cause-effect relationship with EC_{50} exacerbated in the second period studied. Among them, CO_2 and pH are the factors which vary the most.

Main Conclusion

The trend of EC_{50} values recorded in the past two decades has been projected until 2040, revealing a general deterioration of sea urchins' ability to withstand copper toxicity, but also a possible recovery of the health of the population under the most optimistic scenario. As a final consideration, it is still uncertain whether *P. lividus* will be able to adapt to changing climatic conditions or not, and if natural populations will be able to evolve plastic metabolic responses to climate stressors.

Adaptation

The influence of photosymbiosis in *Cassiopea xamachana* regenerative success

Marta Mammone[1], Victoria Sharp[1], Michael Hewitt[1], Mónica Medina[1]

[1] Department of Biology, Pennsylvania State University, University Park, USA

Aim

The regenerative capacity of Scyphozoans (Phylum Cnidaria) has been relatively understudied. The model organism *Cassiopea xamachana* hosts photosynthetic dinoflagellate symbionts in the host's motile amoebocyte cells. A handful of studies have reported regeneration in the polyps of *C. xamachana*, but the mechanisms underlying regeneration have not been fully explored. Despite undergoing drastic developmental changes when symbiotic, the effect of symbiont presence and species on host regeneration has never been explored.

Methods

To explore the influence of photosymbionts, *C. xamachana* polyps were decapitated when aposymbiotic, and symbiotic with both a homologous and a heterologous symbiont species. Observations were carried out with a fluorescence stereo microscope. We counted daily 1) fully detached buds for each polyp, 2) survival and 3) regeneration of the cut stalks and heads. Confocal microscopy was also carried out for counting symbiont numbers. EdU labeling was performed to observe patterns of cell proliferation in regenerating polyps.

Results

Decapitated polyp stalks regenerated a head, but heads did not regenerate the stalk; instead, they produced planuloid buds. In most cases, first signs of regeneration appeared at Day 4 and were completed by Day 14. Both symbiont species and symbiotic state of the host contributed to significant differences in bud count between treatments. No difference in number or location of EdU labeled, cells was observed between algal symbiont or time point.

Main conclusion

The presence of symbionts increased likelihood to regenerate, yet symbiont species did not affect success of regeneration. No blastema or dividing cells were observed, implying cell proliferation is not the primary mechanism behind regeneration in polyps of *C. xamachana*.

Adaptation

Signs of local adaptation by genetic selection and isolation promoted by extreme temperature and salinity in the Mediterranean seagrass *Posidonia oceanica*

Hung Manh Nguyen[1], **Miriam Ruocco**[1], **Emanuela Dattolo**[1], **Federica Paola Cassetti**[2], **Sebastiano Calvo**[2], **AgostinoTomasello**[2], **Lázaro Marín-Guirao**[1,3], **Mathieu Pernice**[4] **and Gabriele Procaccini**[1]

[1] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy.
[2] Dipartimento di Scienze della Terra e del Mare, Università di Palermo, Viale delle Scienze, Ed. 16, 90128, Palermo, Italy.
[3] Oceanographic Center of Murcia, Seagrass Ecology Group, Spanish Institute of Oceanography (IEO-CSIC), C/Varadero, 30740, San Pedro del Pinatar, Murcia, Spain.
[4] Faculty of Science, Climate Change Cluster (C3), University of Technology Sydney, Ultimo, 2007, NSW, Australia.

Abstract

Adaptation to local conditions is known to occur in seagrasses, however, knowledge of the genetic basis underlying this phenomenon remains scarce. Here, we analyzed *Posidonia oceanica* from six sites within and around the Stagnone di Marsala, a semi-enclosed coastal lagoon where salinity and temperature exceed the generally described tolerance thresholds of the species. Sea surface temperatures (SSTs) were measured and plant samples were collected for the assessment of morphology, flowering rate and for screening genome-wide polymorphisms using double digest restriction-site-associated DNA sequencing. Results demonstrated more extreme SSTs and salinity levels inside the lagoon than the outer lagoon regions. Morphological results showed significantly fewer and shorter leaves and reduced rhizome growth of *P. oceanica* from the inner lagoon and past flowering events were recorded only for a meadow farthest away from the lagoon. Using an array of 51,329 SNPs, we revealed a clear genetic structure among the study sites and confirmed the genetic isolation and high clonality of the innermost site. Fourteen outlier loci were identified and annotated with several proteins including those relate to plant stress response, protein transport and regulators of plant-specific developmental events. Especially, five outlier loci showed maximum allele frequency at the innermost site, likely reflecting adaptation to the extreme temperature and salinity regimes, possibly due to the selection of more resistant genotypes and the progressive restriction of gene flow. Overall, this study helps us to disentangle the genetic basis of seagrass adaptation to local environmental conditions and may support future works on assisted evolution in seagrasses.

Adaptation

Exploring stress memory in seagrasses

Jessica Pazzaglia[1], Luca Ambrosino[2], Samuele Greco[3], Koen Verhoeven[4], Gabriele Procaccini[5], Lázaro Marín-Guirao[6]

[1] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, 80121, Naples, Italy, jessica.pazzaglia@szn.it.
[2] Department of Research Infrastructure for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy, luca.ambrosino@szn.it.
[3] Department of Life Sciences, University of Trieste, 34127, Trieste, Italy, samuele.greco@units.it.
[4] Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands, K.Verhoeven@nioo.knaw.nl.
[5] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, 80121, Naples, Italy, gpro@szn.it.
[6] Oceanographic Center of Murcia, Spanish Institute of Oceanography (IEO-CSIC), Murcia, Spain, maringuirao@gmail.com.

Aim

In the Era of Climate change, organisms are experiencing intense and rapid environmental changes worldwide. Coastal marine ecosystems, such as seagrass meadows, are among the most important in the world for providing human services and benefits. However, the ongoing rapid environmental changes are increasing seagrass loss rates, placing them among the most threatened ecosystems on Earth. Seagrasses can reproduce sexually (with flowers and seeds), similarly to their terrestrial relatives, or clonally, producing new identical vegetative individuals. Exploring stress memory in seagrasses through assisted evolution approaches, such as the priming treatment, and integrating multi-level assessments, could revolutionize the restoration plans implemented to date for seagrasses by combining the conservation of these important marine ecosystems with new assisted evolution technologies.

Methods

The thermo-priming treatment was the approach used to assess stress memory in seagrasses. This approach was performed experimentally using indoor aquaria (mesocosms), where plants were treated with first heat stress to induce the priming stimulus. Seagrass performance was analyzed using a multi-level assessment that embraces photo-physiology, transcriptomics, and epigenetics. Global DNA methylation and differentially methylated cytosines were explored for the first time in seagrasses using the reduced representation bisulfite sequencing (RRBS) approach.

Results

The approach was useful to primed *P. oceanica* seedlings as they respond better to the re-occurrence heat stress event. The acquisition of the priming status was accompanied by the largest transcriptomic regulation with respect to not-primed seedlings. DNA methylation results and their integration of transcriptomics could outline key genes involved in the regulation of stress memory.

Main conclusion

Exploring stress memory in seagrasses by using assisted evolution approaches can be advantageous on the one hand to set up specific experimental conditions for enhancing stress tolerance. Integrative responses obtained by merging the NGS with phenotypic responses are essential to investigate specific stress-memory-related genes, including epigenetic regulators.

Adaptation

Female reproductive fluid and male seminal fluid: the non-gametic conflict for post-mating control

Livia Pinzoni[1], Lisa Locatello[2], Clelia Gasparini[1], Maria Berica Rasotto[1]

[1] Department of Biology, University of Padova, Padova, Italy.
[2] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Fano Marine Center, Fano (PU), Italy.

Aim

The competition between sexes to gain control over the fertilization process represents a crucial challenge for all organisms, but particularly for external fertilizers. In this context, marine organisms showcase a variety of male and female adaptations aimed at securing this control.
Growing evidence shows that the non-gametic components released by both males and females can play a major role in influencing the outcomes of fertilization. Seminal fluid was shown to influence paternity success by affecting female mating behaviour and rival males' sperm performance. In turn, female reproductive fluid can differentially influence ejaculates of different males and bias fertilization towards specific partners. However, no one so far has ever considered the consequences of these fluids intermingling for fertilization and sexual selection. In the study I will present we explored this scenario using the grass goby (*Zosterisessor ophiocephalus*), an externally fertilizing fish of the Venice lagoon with territorial-sneaker reproductive tactics, where sneaker males can exploit the territorials' seminal fluid while penalizing territorial sperm performance with their own fluid.

Methods

To test whether female reproductive fluid can rebalance the ejaculate competition in favour of territorial males (the preferred phenotype at the pre-mating level), we used in vitro fertilization with a seminal fluid mixture (territorial + sneaker), and increasing concentrations of female reproductive fluid, to simulate the natural conditions that sperm encounter when swimming towards the eggs.

Results

Our findings revealed a female-driven directional process favouring only territorial males in presence of high concentrations of female reproductive fluid, seemingly mediated by the interaction with the seminal fluids of the different male competitors.

Main conclusion

Through this process, females of the grass goby can discriminate among ejaculates of the different male phenotypes and influence the sperm competition outcome, ultimately reinforcing their pre-mating preference for territorial males and regaining control over the fertilization environment.

Adaptation

SmithRNAs: a new arena for mito-nuclear interaction and coevolution

Federico Plazzi[1], Diego Carli[1], Alessandro Formaggioni[1], Iuri Icaro[1], Marco Passamonti[1]

[1] Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, BO, Italy.

Aim

Evidences of mitochondrially driven RNA interference (RNAi) are recently emerging. Recently, we suggested a new class of small non coding RNAs (sncRNAs) of mitochondrial origin called smithRNAs (Small MITochondrial Highly-transcribed RNAs) in the Manila clam, *Ruditapes philippinarum*, a bivalve species with Doubly Uniparental Inheritance (DUI) of mitochondrial DNA. Among other possible functions, those smithRNAs were predicted to regulate nuclear gene expression in *R. philippinarum* gonad formation, a possibility which has never been suggested before. However, all evidences for smithRNAs were *in silico* predictions, up to now.

Methods

We experimentally tested for significant modifications in methylation/acetylation levels in specimens treated with smithRNAs predicted to target nuclear enzymes connected with such regulatory functions.

Results

SmithRNAs are conserved in the Manila clam and we present significant clues of their functionality *in vivo*. Moreover, it is conceivable that smithRNAs will be found in other eukaryotes as well, maybe linked to functions other than gonad development: in fact, we present evidences that putative smithRNA genes are present in other metazoans' mtDNAs.

Main conclusion

SmithRNAs are good candidates to evolve new functions in the compact mtDNA, because hairpin structures in mitochondrial intergenic regions (needed for correct RNA cleavage) could easily be exapted to evolve RNAi. SmithRNAs make mtDNA a much more complex genome than previously thought, and they represent a new mito-nuclear coevolutionary arena. Actually, the possibility that mtDNA may act as a reservoir of RNAi opens a plethora of new ways for it to interact with the nucleus and significantly raises the level of complexity of mito-nuclear coevolution: for instance, smithRNAs may play a role in reproductive isolation between closely related species.

Adaptation

Comparative transcriptomics between *P. oceanica* e *C. nodosa* populations reveals insights on environmental adaptation mechanisms in seagrasses

Alessia Riccardi[1], Emanuela Dattolo[2], Marco Miralto[1], Gabriele Procaccini[2], Maria Luisa Chiusano[3], Luca Ambrosino[1]

[1] Department of Research Infrastructure for Marine Biological Resources, Stazione Zoologica Anton Dohrn, 80121 Naples, Italy.
[2] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, 80121 Naples, Italy.
[3] Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (NA), Italy.

Aim

Extreme weather events related to the global warming occur more and more frequently and are progressively damaging ecosystems biodiversity. Climate change is slowly disrupting the ecosystems equilibrium also in marine environment, affecting the global distribution of seagrasses. This group of marine angiosperms plays a key role in the coastal areas providing different essential services. Due to their fundamental roles for the structure and conservation of marine biodiversity, studying the strategies adopted by different seagrasses species and how they respond to the environmental factors is essential for predicting their fate in the face of the current environmental changes.

Methods

We analysed the gene expression features related to different populations of two seagrasses (i.e. *Posidonia oceanica* and *Cymodocea nodosa*) coming from different geographical sites in the Mediterranean bioregion with a bioinformatics and comparative approach. Specifically, we provided a comparative transcriptomics analysis using publicly available RNA-seq data and not yet publicly genomic resources. In details, we merged the results obtained from the comparative and transcriptomic analyses to investigate about the expression profile of orthologs and paralogs included in a selected subset of networks.

Results

The differentially expressed genes (DEGs) and the subsequent GO enrichment analyses underlined the involvement in the local adaptation of different biological processes connected with the secondary metabolism (in Cymodocea) and the photosynthesis (in Posidonia). Moreover, the study of the expression profiles of genes in a specific subset of networks including the ortholog DEGs between the two species, suggested that plant's transcriptional profiles have been more deeply influenced by the latitudinal origins rather than local thermal environments.

Conclusion

The findings imply that latitudinal distribution of populations plays a critical role in influencing plant-environment interaction, although remarkable differences exist in the transcriptional and metabolic processes associate to environmental responses between the different species.

Biodiversity

Chairs: **Luca Mirimin** & **Paolo Sordino**

This theme encompasses studies about the variety of co-evolving life, including all genes, species and ecosystems and the ecological processes which they are part of.

Biodiversity

Rebuilding the coral tree of life: Through Ocean Exploration, Museum Specimens, and Genomic Discovery

Andrea Quattrini[1]

[1]Smithsonian National Museum of Natural History.

Abstract

Corals are iconic marine species that engineer habitat for millions of other organisms. Although their importance in enhancing biodiversity in the marine environment is clear, in many depths and regions around the world, they remain under-described, uncharacterized, often inaccurately identified, and/or yet to be discovered. Still, they are threatened by a variety of anthropogenic stressors-from climate change to resource extraction activities. To successfully mitigate the impacts of these stressors, we need fundamental knowledge on how many species there are, and how and where these species are generated, distributed and connected. Therefore, using genomics and integrative taxonomy, coupled with historical collections and ocean exploration, we are beginning to "rebuild" the coral tree of life. By reconstructing this evolutionary framework, we can better document the extent of coral diversity (taxonomic, genetic morphological, functional), while uncovering the historical and contemporary drivers of that diversity, so that we can better inform the likelihood of persistence into the future.

Biodiversity

Bivalves' mitogenomics reloaded: a new backbone for mitochondrial protein annotation in the class Bivalvia

Diego Carli[1], Federico Plazzi[1], Enrico Negrisolo[2], Marco Passamonti[1]

[1] Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy.
[2] Department of Comparative Biomedicine and Nutrition, University of Padova, Padova, Italy.

Abstract

A curated, high-quality annotation of mitochondrial genomes is a key step for manifold downstream analyses, such as phylogenetic inference, mitotranscriptomics, and population structure studies. Although good annotations of mitochondrial genomes are nowadays available for many taxa across the tree of life, this does not hold true for the class Bivalvia. Notably, mitochondria account for some odd peculiarities in this class, including the Doubly Uniparental Inheritance. Therefore, we set up a comparative framework to reconsider the annotation of mitochondrial proteins in bivalves, with special reference to gene boundaries. We used three datasets of mitochondrial genomes, containing species with increasing phylogenetic distance. We sought for reasons of the observed disparities in annotations using different approaches. Here we present some insights into our effort towards a reappraisal of mitochondrial genomics of bivalves, with the double aim of providing a robust mitochondrial gene feature overview of this group as well as suggesting good practices for future annotations.

Biodiversity

Relating anatomical structure and behaviour in the borrowing, clam-predator polychaete *Halla parthenopeia*

Anita Ferri[1], Roberto Simonini[2], Pedro M Costa[3]

[1] Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena MO, Italy.
[2] Department of Life Sciences, University of Modena and Reggio Emilia, Modena MO, Italy.
[3] Associate Laboratory i4HB - Institute for Health and Bioeconomy, UCIBIO - Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal.

Aim

Halla parthenopeia is a borrowing, poisonous, clam-predating polychaeta often used as bait. Besides potential biotechnological applications of the toxins, the feeding apparatus of the worm can offer important insights on the species' adaptation to its habitat. Here we describe the anatomy of *H. parthenopeia* in relation to its lifestyle, chemical defenses and feeding behavior, which rely on the production of different types of mucus.

Methods

Multiple histological and histochemical analyses, both in paraffin and in resin sections, were performed to produce a comprehensive image of the animal's microanatomy.

Results

The tegument is overlayed by a cuticle and is formed by a mucocytes-rich pseudostratified epithelium. Skin mucocytes secrete the mucus that facilitates locomotion and stabilizes the burrow. Between mucocytes we noticed cells with a round soma and dark cytoplasmatic granules. These cells are likely involved in the storage and secretion of hallachrome, the toxic pigment responsible for the purple staining of defensive mucus. The pharynx bears jaws and mandibles that are connected to a single, relatively large, salivary gland. This gland extends from below the esophagus up to one third the length of the animal. This gland produces the feeding mucus, secreted to facilitate opening and digesting clams. The unusual structure of the esophagus, full of different types of mucocytes, may also be associated to the production of gut-lubricating mucins during the feeding.

Main conclusion

The abilities to dig burrows into the sediment, to prey on bivalves and to fence off predators and parasites by *H. parthenopeia* seems to rely on a unique combination of highly specialized anatomical structures. These permit the secretion of multiple type of mucins that convey toxins and enzymes. These features are aligned with the species' complex behavior, altogether representing efficient adaptations ambush preying in shallow marine benthic environments.

Biodiversity

A blue *Tritonia callogorgiae* Chimienti, Furfaro & Taviani, 2020 morphotype (Mollusca, Nudibranchia) reveals unexpected variability in body colour pattern and diet and a complex evolutionary history.

Giulia Furfaro[1,2,3], Paolo Mariottini[4], Egidio Trainito[5], Giovanni Chimienti[3,6], Jeroen A.J.M. van de Water[7,8], Laurent Ballesta[9], Julie Deter[9,10]

[1] Department of Biological and Environmental Sciences and Technologies - DiSTeBA, University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. giulia.furfaro@unisalento.it.
[2] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy.
[3] CoNISMa, Rome, Italy.
[4] Department of Science, University of "Roma Tre", Viale G. Marconi 446, 00146 Rome, Italy. paolo.mariottini@uniroma3.it.
[5] Genoa Marine Centre-Stazione Zoologica Anton Dohrn, Istituto Nazionale di Biologia, Ecologia e Biotecnologie Marine, Villa del Principe, Piazza del Principe 4, 16126 Genoa, Italy. et@egidiotrainito.it.
[6] Department of Biosciences, Biotechnologies and Environment (DBBA), University of Bari Aldo Moro, Italy. giovanni.chimienti@uniba.it.
[7] Centre Scientifique de Monaco, 8 Quai Antoine 1er, MC 98000, Monaco, Principality of Monaco. jeroen.van.de.water@nioz.nl.
[8] Department of Estuarine & Delta Systems, Royal Netherlands Institute for Sea Research, Korringaweg 7, 4401 NT, Yerseke, The Netherlands.
[9] Andromède océanologie, 7 place Cassan – Carnon plage, 34130 Mauguio, France. contact@andromede-ocean.com
[10] Université de Montpellier – UMR MARBEC, campus triolet, place E. Bataillon, 34000 Montpellier, France. julie.deter@umontpellier.fr.

Aim

Deep-sea coral habitats host a complex associated fauna that has not been fully revealed yet. The nudibranch *Tritonia callogorgiae* Chimienti, Furfaro & Taviani, 2020, was recently described from deep *Callogorgia verticillata* (Pallas, 1766) forests and nowadays found exclusively associated to this octocoral. This tritoniid shows an orange background colour scattered with white and orange gills with white/transparent apical parts. In 2021, during the *Gombessa 6* expedition, specimens resembling *T. callogorgiae* but showing an atypical coloration were observed and photographed on *Paramuricea clavata* (Risso, 1827) off the coast of Corsica. These specimens had dark blue gills and light blue edge of the sheath of the rhinophores. To unravel whether this blue morphotype could mask hidden diversity, five specimens were sampled for molecular comparison.

Methods

Six blue and two orange specimens, collected respectively on *P. clavata* and *C. verticillata* at 96 and 117 m depth, were photographed and stored. DNA was extracted and DNA-Barcoding, Bayesian Inference and Maximum Likelihood analyses were performed using Cytochrome C Oxidase subunit I, 16S rRNA and histone H3 molecular markers. A broader sequence dataset, including *T. callogorgiae* topotypical material and other Tritoniidae species, was considered.

Results

DNA-Barcoding confirmed the blue specimens were indeed a morphotype of *T. callogorgiae*, despite the striking colour difference. Besides, phylogenetic analyses revealed a complex evolutionary history within this species, which is provisionally kept in *Tritonia* even though morphological features suggest it may possibly belong to a separate genus. Finally, a new trophic association with *P. clavata*, is reported.

Main conclusion

Results showed that *Tritonia callogorgiae* has an extremely variable body colour pattern and diet. Furthermore, a complex phylogeny was revealed among this species and other Tritoniidae that should be elucidated in the future. Finally, evidence suggest the possible correlation between prey and colour variability that deserves future investigations.

Biodiversity

An updated phylogeny of the family Opheliidae (Annelida) based on complete mitogenomes

Leandro Gammuto[1], Joachim Langeneck[2], Alberto Castelli[1], Giulio Petroni[1]

[1] Dipartimento di Biologia, Università di Pisa, via Derna 1, 56126 Pisa, Italy.
[2] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), U.L.R. di Lecce, c/o DiSTeBA, campus Ecotekne, strada provinciale Lecce-Monteroni, 73100 Lecce, Italy.

Aim

In the last years an approach based on multiple molecular markers has been increasingly used in phylogenetic studies. The usage of whole mitochondrial genomes has already been shown to be of great help in addressing phylogenetic and systematic issues at various levels (e.g. depicting cryptic species, discriminate between subspecies or investigate recent evolutionary phenomena). Phylogenies based on whole mitochondrial genomes usually allow for a higher overall support of nodes than traditional phylogenies based on a few loci, and with much lower costs compared to -omics approaches. In this framework annelids are a particularly interesting group, as they were considered to be uniform in terms of mitogenome size and synteny, assumptions that were later challenged by recent studies.

Methods

Total DNA material of seven species of Opheliidae (covering the majority of the currently valid genera) was amplified via whole-genome amplification (WGA) method, and then processed via Illumina technology. Obtained reads were assembled with SpADES to obtain the complete mitochondrial genome.

Results

Mitochondrial genomes of the family Opheliidae do not show a wide variation in size, being overall between 15,000 and 17,000 base pairs. Conversely, the gene order shows a wide variation, and four main orders can be identified, one of which corresponding to the putative annelid ground pattern. The obtained phylogenetic analysis only partially confirms previous reconstructions, allowing to identify three clades, corresponding to different morphologies and with a coarse correspondence with gene order patterns.

Main conclusion

Mitochondrial genomes revealed themselves a promising and powerful tool to disentangle intra-familiar relationships and solve systematic issues, being able to infer relationships inside the order with good statistical support. Synteny in Opheliidae shows a good correspondence with the main clades retrieved, even if this result cannot be extended to other annelid families.

Biodiversity

Molecular biodiversity assessment of nekton in the Tyrrhenian deep sea

Martina La Torre[1], Alex Cussigh[2], Alessia Cariani[2], Federica Costantini[2], Alice Ferrari[2], Silvia Franzellitti[2], Alberto Pallavicini[3], Federica Piattoni[2], David Stankovic[4], Sergio Stefanni[5]

[1] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Roma, Italy; martina.latorre4@unibo.it.
[2] Department of Biological, Geological and Environmental Sciences, University of Bologna, Ravenna, Italy; alex.cussigh2@unibo.it, alessia.cariani@unibo.it, alice.ferrari6@unibo.it.
[3] Stazione Zoologia Anton Dohrn, Napoli, Italy; pallavic@units.it.
[4] National Institute of Biology, Ljubljana, Slovenia; david.stankovic@nib.si.
[5] Department of Life Sciences, University of Trieste, Trieste, Italy; sergio.stefanni@szn.it.

Aim

The deep sea is the largest biome on earth and yet the least known. Our poor knowledge of its remote habitats reduces the possibility to understand and protect such an important environment. In this study, molecular tools and bioinformatic pipelines were implemented to unravel nekton biodiversity in the poorly investigated Dohrn Canyon in the Tyrrhenian Sea, with a focus on cephalopods and fish species. An eDNA-based metabarcoding approach has been applied to water and sediment samples collected at different depths in the Dohrn Canyon to study cephalopods and fish assemblages.

Methods

Total eDNA was successfully extracted and amplified for the 18S (De Jonge et al. 2021), 12S and COI (Valentini et al. 2016, Miya et al. 2015). Cleaned PCR products were used for library preparation and sequenced. Data derived from the water and sediment matrices (between ca. 280,000 and 17,356,008 raw reads) were cleaned, filtered, and assigned to the lowest possible taxonomic level.

Results

Among the main cephalopod taxa detected *Heteroteuthis* sp., *Loligo* sp. and *Eledone cirrhosa* were the most abundant across all the stations, while concerning teleost, preliminary results highlighted the presence of some deep-sea fishes like *Lampanyctus pusillus*, *Mobula* sp. and *Electrona* sp., among others.

Main conclusion

Despite some methodological constraints, this study represents one of the few attempts applying the emerging eDNA approaches to a deep-sea environment in the Mediterranean Sea. Most importantly, we described the Dohrn Canyon megafaunal biodiversity, with an important and up-to-date focus on the cephalopods community.

Biodiversity

Environmental diversity, distribution and evolution of microbial metal ion transport systems

Flavia Migliaccio[1], Martina Cascone[1], Davide Corso[1], Deborah Bastoni[1], Francesco Montemagno[1], Matteo Selci[1,2], Feliciana Oliva[1], Gabriella Gallo[1], Alessia Bastianoni[1,3], Annarita Ricciardelli[1], Bernardo Barosa[1], Luca Tonietti[1,4,5], Monica Correggia[1], Luciano di Iorio[1], Costantino Vetriani[2,6], Peter H. Barry[6], Rebecca L. Tyne[6], Karen G. Lloyd[7], Gerdhard L. Jessen[8], Agostina Chiodi[9], Maarten J. de Moor[10,11], Carlos J. Ramirez[12], Matt Schrenk[13], Angelina Cordone[1], Donato Giovannelli[1,2,6,14,15]

[1] Department of Biology, University of Naples Federico II, Naples, Italy.
[2] Department of Marine and Coastal Science, Rutgers University, New Brunswick, NJ, USA.
[3] Instituto de Ciencias de la Tierra, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.
[4] University of Naples Parthenope, Naples, Italy.
[5] INAF-OAC, Osservatorio Astronomico di Capodimonte, Naples, Italy.
[6] Woods Hole Oceanographic Institution, Woods Hole, MA, USA.
[7] Microbiology Department, University of Tennessee, Knoxville, TN, USA.
[8] Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile & Center for Oceanographic Research COPAS COASTAL, Universidad de Concepción, Valdivia, Chile.
[9] Instituto de Bio y Geociencias del Noroeste Argentino (IBIGEO, CONICET-UNSa), Salta, Argentina.
[10] Observatorio Vulcanológico y Sismológico de Costa Rica, Universidad Nacional, Heredia, Costa Rica.
[11] Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM, USA.
[12] Servicio Geológico Ambiental, Heredia, Costa Rica.
[13] Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA.
[14] Institute of Marine Biological Resources and Biotechnologies, National Research Council, Ancona, Italy.
[15] Earth-Life Science Institute, Tokyo Institute for Technology, Tokyo, Japan.

Aim

Transition metals such as iron, zinc, copper, molybdenum, nickel, manganese, tungsten and cobalt play crucial roles in microbial metabolism. They function as essential cofactors for critical enzymes involved in electron transport, and can affect cell growth and viability by disrupting cell components integrity. Microorganisms have developed complex transport systems to meet their demands for each metal, simultaneously maintaining the homeostasis of multiple cations within a cell.

The present study aims at describing the diversity and distribution of microbial metal transport systems across diverse geothermal environments, with a specific focus on shallow water hydrothermal vents and terrestrial deeply sourced seeps. In these ecosystems microbial diversity and metabolism are tightly linked to the elements provided by the water-rock interactions, providing an excellent model system in which to investigate the diversity of microbial metal transport systems.

Methods

We performed shotgun metagenomics on geofluids from around 200 thermal features across Costa Rica, Chile, Argentina, Panama, Iceland, and Italy, following a modified version of *KBase* and a custom workflow. The diversity of microbial metal transport genes was assessed via functional annotation of sequencing reads using *mi-faser* with a tailored database including metal transport genes sequences.

Results

The terrestrial and marine environments in this study present wide ranges of physico-chemical variables, and the diversity of genes encoding microbial metal transport systems appears to be influenced by this geochemistry.

Main conclusion

The study of the diversity of metal transport systems is crucial to investigate the interactions between microorganisms and ecosystems, thus their metabolic evolution.

Biodiversity

Cryptic species in time and space: an assessment of cryptic diversity within eight nominal species of Hydrozoa (Cnidaria)

Maria Pia Miglietta[1]

[1] Texas A&M University at Galveston, Galveston, TX, USA.

Abstract

Although cryptic species have routinely been uncovered using barcoding techniques, sympatric cryptic species appearing in the plankton at different times of the year have not yet received adequate attention. This is primarily due to the lack of the necessary temporally dense, long-term sampling that can uncover them. Using barcoding techniques, we analyze the phylogeny of eight species of Hydrozoa (Cnidaria) commonly found in the Gulf of Mexico (GoM) and localities worldwide. We created a unique dataset for each species, combining sequences from specimens collected weekly for a year in the GoM and sequences collected worldwide. Using these datasets, we investigate the presence of sympatric cryptic species appearing in seasonal/temporal gradients (within the GoM) and cryptic species worldwide. We show that eight species of Hydrozoa with wide geographical distribution are composed of cryptic species that span geographically across the globe and seasonally within the GoM. This bi-dimensional cryptic diversity - both in time and space – is essential for a deeper understanding of processes of evolution, biogeography, and dispersal in the seas and more realistic biodiversity assessments.

Biodiversity

A first look at soft corals' toxins: diversity and evolution of venom in the white seafan *Eunicella singularis*

Maria Vittoria Modica[1], Serena Leone[1], Marco Gerdol[2], Sebastien Dutertre[3]

[1] Dept. BEOM Stazione Zoologica Anton Dohrn, Italy.
[2] Dept. of Life Sciences, University of Trieste, Italy.
[3] Institute for Biomolecules Max Mousseron, CNRS, France.

Aim

Cnidaria is one of the most ancient lineages of Metazoa and is characterized by the production of venom in specialized cells. The acquisition and diversification of venom plays a key role in the evolutionary success of the entire phylum. However, research on Cnidaria venom has been mostly focused on sea anemones, Anthozoans belonging to the subclass Hexacorallia. Notably, scarce information is available on the venom produced by the sister subclass of Octocorallia, or soft corals, and it mostly pertains to their small molecules content. Polyps of soft coral colonies are equipped with nematocyst-rich tentacles used to feed on zooplankton and to deter potential predators. The lack of information regarding polypeptide toxins of soft corals is a significant bias towards the understanding of the evolutionary history of Anthozoan venom toxins.

Methods

We used a combined transcriptomic-proteomic approach to investigate the venom of the white seafan *Eunicella singularis*, a Mediterranean soft coral inhabiting rocky bottoms at 20-30 m depth. We isolated venom polypeptides through selective chemical extraction of the nematocysts and we matched MS/MS proteomic data against a whole body assembled transcriptome. Finally, we performed comparative genomic and phylogenetic analyses of relevant proteins, to gain insights on their evolutionary history.

Results

We identified potential cytolytic and neurotoxic proteins in the venom of *E. singularis*, finding similarities with well characterized families of cnidarian toxins, such as the small cysteine rich peptides (SCRIPs). In addition, we detected uncommon polypeptides with novel cysteine frameworks. While some of these toxins seem to be more widespread across soft corals or either within all Anthozoa, other are restricted to specific Octocorallia clades. Homology models and surface electrostatic potentials were analyzed for the previously uncharacterized toxins, to understand their possible mode of action in comparison to well characterized proteins.

Main Conclusions

Our results provide the first description of the toxins from a soft coral's venom, highlighting their potential activity and expanding our understanding of the evolutionary history of venom protein families in Anthozoa.

Biodiversity

Evolutionary patterns in octocoral-microbiota associations

Camille Prioux[1], Romie Tignat-Perrier[1], Christine Ferrier-Pagès[1], Antonin Guilbert[2], Stéphanie Reynaud[1], Cécile Rottier[1], Julie Deter[2], Laurent Ballesta[2], Denis Allemand[1], Jeroen Van De Water[1,3]

[1] Centre Scientifique de Monaco, 8 Quai Antoine 1er, MC 98000, Monaco, Principality of Monaco.
[2] Andromède océanologie, 7 place Cassan – Carnon plage, 34130 Mauguio, France.
[3] Department of Estuarine & Delta Systems, Royal Netherlands Institute for Sea Research, Korringaweg 7, 4401 NT, Yerseke, The Netherlands.

Abstract

Corals are the foundational species of coral reefs and coralligenous ecosystems. Their success may be linked to their symbioses with microorganisms, which play an important role in the nutrition and health of their host. Therefore, the coral host and its symbionts are considered an entity: the holobiont. But whether there is an evolutionary link between corals and their microbiomes has not been fully investigated. While indications of phylosymbiosis in Scleractinian hexacorals exist, little is known about the holobionts of Alcyonacean octocorals. Here, we used *16S rRNA* gene amplicon sequencing to explore the composition of the bacterial communities associated with 14 Alcyonacean species (belonging to 10 genera from 5 (sub)orders) collected from the mesophotic zones of the Mediterranean Sea and Red Sea. The low levels of disturbance in mesophotic ecosystems enabled us to study potential evolutionary links (e.g., phylosymbiosis and cophylogeny) between these corals and their microbiota. Clear differences were, however, observed between corals from the Mediterranean Sea and the Red Sea. The low diversity and consistent dominance of *Endozoicomonadaceae* and/or *Spirochaetaceae* in the communities of Mediterranean octocorals, suggested that Mediterranean octocorals could have evolved in parallel with their microbiota. Phylosymbiotic signals were indeed detected and cophylogeny in associations between several bacterial strains belonging to *Endozoicomonadaceae* or *Spirochaetaceae* and coral species were identified. Conversely, phylosymbiotic patterns were not evident in Red Sea octocorals, likely due to the high bacterial taxonomic diversity in their microbiota, but cophylogeny in associations between certain coral and bacterial species were observed. Particularly noteworthy were the associations with *Endozoicomonadaceae*, suggesting a plausible evolutionary link that warrants further investigations with expanded datasets to uncover potential underlying patterns. Overall, our findings emphasize the importance of *Endozoicomonadaceae* and *Spirochaetaceae* in coral symbiosis and the significance of exploring host-microbiome interactions in mesophotic ecosystems for a comprehensive understanding of coral-microbiome coevolution.

Biodiversity

From 2 to 12 or more: how many *Myxicola* species are out there?

Matteo Putignano[1,2], Joachim Langeneck[2], Andrea Toso[1,2], Adriana Giangrande[1,2,3]

[1] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), Università del Salento, strada provinciale Lecce-Monteroni, 73100 Lecce, Italia.
[2] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), U.L.R. di Lecce, c/o DiSTeBA, Università del Salento, strada provinciale Lecce-Monteroni, 73100 Lecce, Italia.
[3] National Biodiversity Future Center (NBFC), 90133 Palermo, Italia.

Aim

A recent work uncovered an unexpected diversity inside the genus *Myxicola* Koch in Renier in Meneghini, 1847 (Sabellidae, Annelida) in the Mediterranean Sea. The analysis of new samples from deep coralligenous and mesophotic assemblages highlighted the occurrence of potential new lineages within this genus. The increase in the number of species belonging to this genus calls for the need of a better understanding of the phylogenetic relationships occurring among them.

Methods

Morphological analysis employed more than 40 different characters, to differentiate between taxa; in comparison with previous studies, the possibility to work on fresh material allowed us to detect characters appreciable only on live specimens. In addition, two mitochondrial markers (16S rDNA and COI) were used for molecular characterization and phylogenetic reconstruction.

Results

Eight different lineages were recovered within the genus *Myxicola* in the Mediterranean Sea alone, while 4 additional lineages were obtained from public datasets (GenBank and BOLD). Two Mediterranean lineages resulted clearly distant from the remaining ones, allowing to retrieve a closer relationship with the genus *Acromegalomma* and suggesting that they should be considered as a different, new genus. Morphological results were consistent with molecular one, concerning the shape of abdominal uncini, radiolar skeleton and structure of lips, as well as ecological differences.

Main conclusion

The possibility to work on environments scarcely known allowed the detection of an unforeseen biodiversity, falling into a genus previously characterized by few, cosmopolitan species. Although polychaetes have been recently studied for their abundant cryptic complexes, a vastly overlooked diversity still lies within this group, suggesting a largely underrated species diversity.

Biodiversity

Unraveling the Evolution and Function of the Retinoic Acid Signaling in the Mollusk *Mytilus galloprovincialis*

Federica Salatiello[1,2], Angelica Miglioli[3], Rosa Maria Vitale[4], Valeria Tarallo[5], Claudia La Vecchia[1,6], Rémi Dumollard[3], Michael Schubert[3], Alberto Pallavicini[1,2], Marco Gerdol[2*], Enrico D'Aniello[1]

[1] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy.
[2] Department of Life Sciences, University of Trieste, Trieste, Italy.
[3] Sorbonne Universités/CNRS, Institut de la Mer à Villefranche, UMR7009, LBDV, Villefranche sur-mer, France.
[4] Institute of Biomolecular Chemistry, National Research Council of Italy, Pozzuoli, Italy.
[5] Institute of Genetics and Biophysics "Adriano Buzzati Traverso", CNR, Naples, Italy.
[6] TIGEM - Telethon Institute of Genetics and Medicine | Via Campi Flegrei, 34 | 80078 Pozzuoli (NA), Italy.

Aim

Retinoic acid (RA) plays several essential roles during embryogenesis and throughout adult life in Metazoan. In vertebrates, RA controls the transcription of target genes through binding to retinoic acid receptors (RAR). To date, very little is known about RA function in invertebrates. Recent findings reported that Lophotrochozoan RAR lost the ability to bind RA due to the accumulation of multiple mutations in the ligand binding domain (LBD). Here, we use the bivalve *Mytilus galloprovincialis* (*Mg*) to provide new insights into the role and evolution of RA signalling in molluscs.

Methods

Genomic screening and phylogenetic inference were used to identify ortholog sequences to vertebrate RA-related proteins. Later, a detailed analysis of the LBD domain of the *Mg*RAR revealed key aminoacidic changes with vertebrates. Molecular docking and dynamics simulations together with functional assay were conducted to test *Mg*RAR ability to respond to RA. Finally, embryos were treated with different doses of RA and with specific inhibitors.

Results

RA machinery toolkit genes are conserved with Vertebrates. However, functional *in vitro* luciferase assay revealed the lack of activation of *Mg*RAR in the presence of RA, even if, *in vivo* treatments of embryos with this compound or with specific inhibitors resulted in many developmental defects.

Main Conclusions

Although our preliminary results suggest that *Mytilus* RAR do not bind RA, we cannot fully exclude the contrary given the limitation of our Luciferase assay system. Therefore, currently, we are using other approaches before to confirm the inability of the *Mg*RAR to be responsive to RA. Furthermore, ongoing RNAseq analyses will help better to explore the potential RA role during Mg embryogenesis.

Biodiversity

Environmental DNA metabarcoding as a tool for coastal monitoring: a case study in the Southern Adriatic Apulian coastal zone

Alice Tagliabue[1], Giulia Furfaro[1,2], Stefano Piraino[1,2]

[1] Department of Biological and Environmental Sciences and Technologies- DiSTeBA, University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. alice.tagliabue@unisalento.it; giulia.furfaro@unisalento.it; stefano.piraino@unisalento.it.
[2] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy.

Aim

Attention for rapid assessment of biodiversity and ecosystem integrity has been greatly revived by the advent of environmental DNA (eDNA) metabarcoding, a quick, cost-effective, and non-invasive molecular methodology. Traditionally targeted by standard parameters and/or indexes foreseen by the EU Water Directive, so far, the biological quality of the Apulian waters has never been explored using eDNA. Thus, a case study has been carried out, focused on a coastal zone subject to different levels and types of anthropic impacts.

Methods

18S and COI markers were used to screen for differences in the structure and organization of benthic macroinvertebrate communities at three stations near Brindisi (Apulia) in the Southern Adriatic coast: i) the Torre Guaceto Marine Protected Area as control site, and two impacted sites namely ii) the nearshore zone adjacent to the Brindisi main harbour, and iii) the proximate coastal zone offshore the ENEL thermoelectric power plant Federico II (Cerano). A user-friendly standardized protocol was implemented to obtain replicable and statistically reliable results. At each site, three sampling stations were located along a linear transect at 100, 500 and 1000m distance from the shoreline. Water and sediment samples were collected by SCUBA diving, in eight points at each station and sediment samples were sub-sampled three times. A total of 576 eDNA were extracted and a two step-PCR were performed to build the libraries for Illumina NovaSeq™ sequencing.

Results

Bioinformatic analysis of reads and statistical study of alpha- and beta-diversity produced robust data of the community structures occurring across the investigated marine area, seeking for changes in benthic assemblages due to distance from the coast, anthropogenic impacts, and seasonality.

Main conclusion

Our preliminary results will be reported here, witnessing the eDNA approach can be powerful to providing information on the welfare of natural marine environments, and calling attention for future applications of the same technique across multiple areas.

Biodiversity

New insights into the systematics of the family Pyuridae (Ascidiacea: Stolidobranchia): integrative taxonomy and molecular phylogeny of the Mediterranean species

Riccardo Virgili [1,2], Xavier Turon [3], Angelo Fontana [2,4], Fabio Crocetta [1]

[1] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Napoli, Italy.
[2] Department of Biology, University of Naples "Federico II", Napoli, Italy.
[3] Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Blanes, Spain.
[4] Bio-Organic Chemistry Unit, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Napoli, Italy.

Aim

Ascidians (Chordata: Tunicata) account for more than 3,000 species worldwide, showing an impressive, albeit often understudied, diversity. The Mediterranean ascidian fauna is commonly considered as well-characterized, although it has been only partially investigated through modern approaches. We hereby carried out the first integrative study focusing on the family Pyuridae (Ascidiacea: Stolidobranchia) in the Mediterranean Sea, clarifying the taxonomic status and systematic position of the investigated species within the complex stolidobranch phylogeny.

Methods

An integrative approach based on morphological examination and single and multi-locus phylogenetic reconstructions was done on specimens collected from various localities in the Mediterranean Sea. In particular, the mitochondrial genes COI and 16S rRNA, and the nuclear genes 18S rRNA and 28S rRNA, were used. A preliminary phylogenetic reconstruction encompassing Pyuridae and its related groups (Styelidae and Molgulidae) was carried out on the basis of available loci mined from transcriptomic and WGS published data.

Results

The family Pyuridae resulted to be paraphyletic, in agreement with published literature. Taxonomic mismatches and cryptic diversity were first highlighted in the genera *Microcosmus* Heller, 1877 and *Pyura* Molina, 1782.

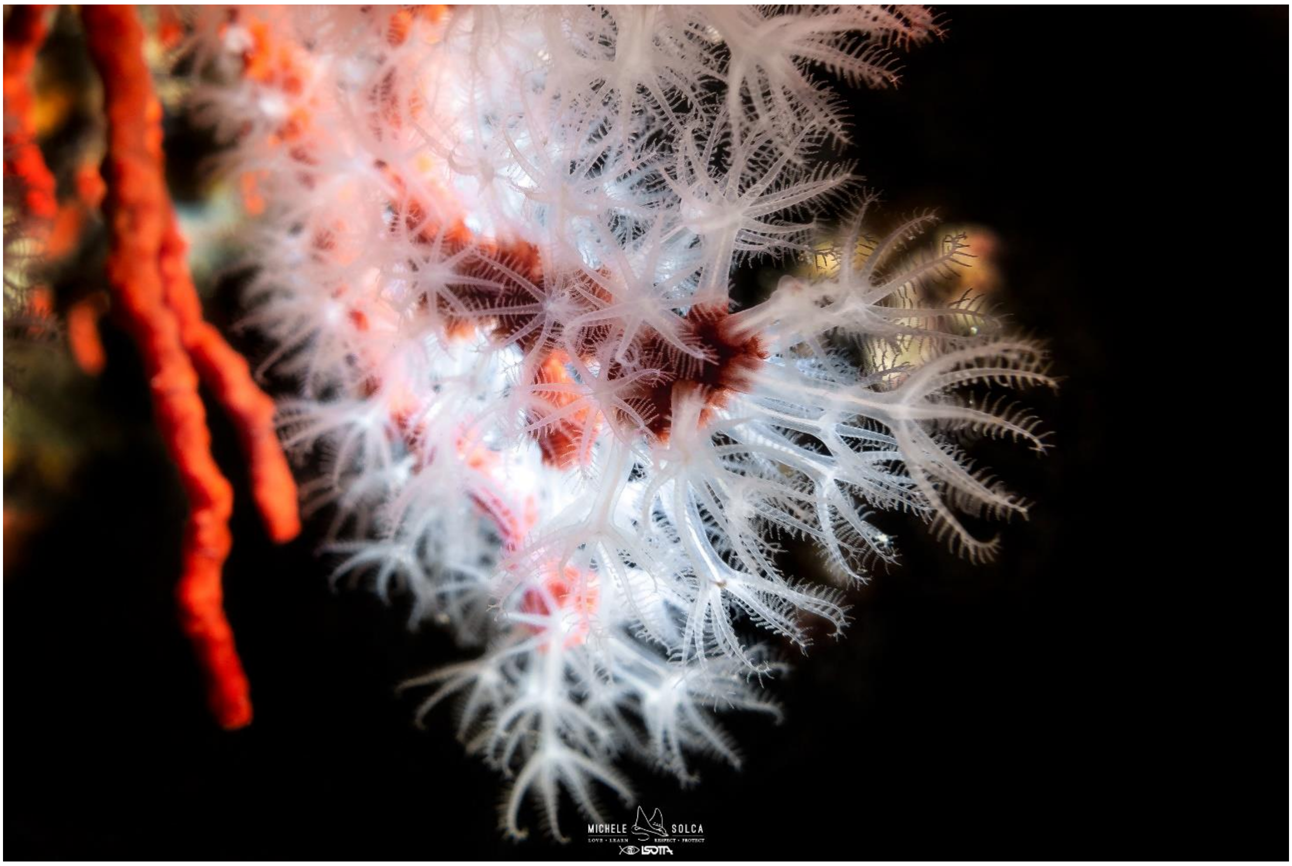
Main conclusions

This contribution provides a first baseline for a general revision of the family Pyuridae and its representatives in the Mediterranean Sea. As these species include important ecosystem engineers, commercially valuable species, renowned worldwide invaders, and important sources of bioactive marine natural products, it also highlights the need of multipronged approaches to investigate their diversity and ecology.

Conservation

Chairs: **Lucia Bongiorni** & **Emiliano Trucchi**

This theme addresses the estimation and preservation of the evolutionary potential of marine ecosystems to face current and future environmental challenges, with a focus on genetic and genomic approaches.



Conservation

The World According to Sharks: Lessons on surviving climate change from Earth's oldest predator

Toby S. Daly-Engel[1]

[1] Department of Ocean Engineering and Marine Sciences, Florida Institute of Technology, Melbourne, FL, United States
tdalyengel@fit.edu

Abstract

Elasmobranchs (sharks, skates, and rays) are one of the oldest animal groups on the planet, having first appeared over 450 million years ago, before trees and before Saturn had rings. Important evolutionary innovations such as closing jaws and live birth first appeared in ancestors of sharks and their relatives, as well as the prototype cellular mechanisms of our own mammalian adaptive immune system. As top predators, extant sharks play a vital role in preserving the health of the world’s oceans, but have among the slowest growth and reproductive rates in nature- more like humans or whales than like other fishes- and as a result are particularly vulnerable to disturbance. Alarmingly, recent studies show that shark abundance has declined >70% in just the past 50 years, and over a third of remaining species are at risk of extinction. Despite major conservation concern, relatively little is known about the biological factors influencing the long-term viability of shark populations, including how they adapt to environmental challenges. Now, global warming is altering the marine environment in unprecedented ways, and many species are facing an uncertain future. In this talk, we explore what an animal lineage that has outlasted five major global extinctions can teach us about surviving climate change, and how scientists are harnessing the power of their ancient immune systems to preserve shark populations into the future.

Conservation

Pioneering metabarcoding of stranded cetaceans: Stomach contents unleashed!

Sofia Albrecht[1], Sinéad Murphy[1], Cyril Gallut[2], Jean-Luc Jung[2], Orla Slattery[1], Cóilín Minto[1], Emer Rogan[3], Luca Mirimin[1]

[1] Marine and Freshwater Research Centre, Department of Natural Sciences, School of Science and Computing, Atlantic Technological University, Galway, Ireland.
[2] Muséum National d'Histoire Naturelle, CNRS, Institut de Systématique, Évolution, Biodiversité ISYEB, Sorbonne Université, Sorbonne, France.
[3] School of Biological, Earth & Environmental Sciences (BEES), University College Cork, Cork, Ireland.

Aim

Overfishing has altered marine food webs in European waters, including top predators' trophic ecology. The common dolphin is one of the most abundant cetaceans in the North-east Atlantic, playing a key functional role. A decrease in body conditions has been observed in the past, suspected to be related to changes in fish stocks. The aim of this study was to advance the study of common dolphins' dietary shifts by assessing the feasibility of molecular approaches (metabarcoding) for diet analysis from stomachs of dead stranded animals.

Methods

Stomach content analysis was carried out from 84 dead stranded individuals. Dissection protocols have been adapted to allow the collection of environmental prey DNA alongside with hard parts for conventional identification of contents and parasites. Subsequently, DNA was extracted and processed for Illumina sequencing using teleost (fish) and cephalopods (squid) specific markers for 16S and COI region.

Results

The method applied was able to successfully provide sequencing data, unravelling diet composition of a high proportion of stomach contents originating from animals in different conditions. Multiplexing markers served well in generating output while conserving resources and the efficiency of the different markers will be discussed. During analysis, the detection of a range of species of fish and squid originating from different taxa is expected.

Main conclusion

Identifying diet composition and trophic ecology of top predators through metabarcoding is feasible using samples of stranded cetaceans. A successful protocol of sample treatment has been developed based on the adaption of previous employed techniques of Illumina Sequencing and is recommended for future use with advantages in comparison to a previous first approach in literature. Results of the analysis will be used to complete conventional diet analysis to identify dietary shifts and to compare conventional and molecular stomach content analysis.

Conservation

Genomic Erosion on Fin Whales (*Balaenoptera physalus*) in the Mediterranean Sea and the Sea of Cortez Populations

Roberto Biello[1,2], Alessio Iannucci[1,3], Silvia Fuselli[2], Maria Cristina Fossi[4], Cristina Panti[4], Jorge Urbán Ramírez[5], Annalaura Mancia[2]

[1] Department of Biology, University of Florence, Sesto Fiorentino, Italy.
[2] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy.
[3] National Biodiversity Future Center, Palermo, Italy.
[4] Department of Environmental, Earth and Physical Sciences, University of Siena, Siena, Italy.
[5] Departamento de Ciuencias Marinas y Costeras, Universidad Autónoma de Baja California Sur, La Paz, Mexico.

Abstract

Population bottlenecks are likely to have genome-wide negative consequences due to increased genetic stochasticity and lower efficacy of selection, resulting in the loss of adaptive variants and an increase in the frequencies of deleterious variants. The worldwide fin whale (*Balaenoptera physalus*) was intensively exploited by commercial whaling, and present populations differ significantly in abundance, connectivity, and history, providing a unique opportunity to analyse the impact of past demography on genomic composition. Despite their global presence, fin whales rarely traverse the equatorial areas, hence their range is defined by the equator and major landmasses. In some productive warm-water regions, smaller resident populations can be found in semi-enclosed seas like the Mediterranean and the Sea of Cortez (Gulf of California, Mexico). Here, we provide a new reference genome and examine genomic erosion on fin whales from the two semi-enclosed basins. In particular, we sequenced and examined for the first time the genomes of 13 fin whales from the Mediterranean Sea and inferred the degree of genomic isolation from the related North Atlantic population. For comparison, we sequenced and analysed seven genomes from the Sea of Cortez, known for its geographic and genetic isolation, and contextualized our newly generated data with samples from the North Atlantic (Iceland and Svalbard), already available in the literature. Our results support that fin whales from the Mediterranean Sea are not isolated but appear to be part of a larger population with fin whales from the North Atlantic. We revealed an overall genomic erosion in the small and isolated Sea of Cortez population, with lower levels of genetic diversity and longer runs of homozygosity, while no such genomic erosion was detected in the Mediterranean Sea population. More investigations are focusing on the genetic load of these populations, which is expected to improve current conservation policies and management. These findings will provide a more comprehensive picture of the status and trends of fin whales and help to guide successful conservation efforts.

Conservation

Integrating ecological and genomic insights for monitoring conservation status and trends in seahorses

Luciano Bosso[1], Melisa Olave[2,3], Maeva Gabrielli[4], Roberto Biello[4,5], Josefin Stiller[6], Ralf Schneider[7], Andrea Benazzo[4], Giorgio Bertorelle[4], Francesca Raffini[8]

[1] Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy.
[2] Argentine Dryland Research Institute of the National Scientific and Technical Research Council (IADIZA-CONICET), 5500 Mendoza, Argentina.
[3] Faculty of Exact and Natural Sciences, National University of Cuyo, 5500 Mendoza, Argentina.
[4] Department of Life Sciences and Biotechnology, University of Ferrara, 44121 Ferrara, Italy.
[5] Department of Biology, University of Florence, 50019 Sesto Fiorentino, Italy.
[6] Villum Centre for Biodiversity Genomics, University of Copenhagen, Denmark.
[7] Marine Evolutionary Biology, Zoological Institute, Kiel University, 24118 Kiel, Germany.
[8] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy.

Abstract

Seahorses (*Hippocampus* spp.) are flagship animals inspiring numerous conservation programs. They are the first marine genus to be fully listed on the Convention on International Trade in Endangered Species (CITES) Appendix II due to their substantial vulnerability to overexploitation and habitat loss. The peculiar life history of these fishes has been widely addressed through evolutionary and ecological analyses. Yet, no study has integrated current knowledge to approach species-based conservation status, including trends in abundance, diversity, and threats, hindering seahorses' worldwide effective assessment and management. Here, we bridge these gaps by taking advantage of the available geographic, ecological and genomic (ultra-conserved elements and whole genomes) data in the *Hippocampus* species at a global scale, and present the most comprehensive study of the seahorses' conservation status to date. Specifically, we explore species distributions and perform ecological niches modelling, phylogenomic mapping, comparative genomic applications, demographic inferences, and conservation gap analyses. We consider their evolutionary history, demographic changes and genomic erosion including genetic load, which have been largely disregarded by current conservation policies. We investigate the degree and type of protective measures currently granted to seahorses, and the biological and ecological factors that are contributing to their existing and future extinction risk. Our results raise questions on whether the current conservation indicators and practices are effective in preserving the present diversity and function of these iconic fishes, as well as their future evolutionary potential and ecological resilience. These insights provide a broad, more complete picture on the status and trends of seahorses and inform successful conservation initiatives. Finally, as more diverse datasets become available in the light of the "omics" and "open-access" era, we discuss the unprecedented opportunities to address interdisciplinary studies required for proper research-based conservation, assessment, and management of biodiversity.

Conservation

Counting stars: local and global projections of gastropod diversity

Giacomo Chiappa[1], Luigi Romani[2], Giulia Fassio[1], Dario Zuccon[3], Daniele De Angelis[1], Daniele Pagli[4], Yuri Kantor[5], Ellen Strong[5], Paolo G. Albano[7], Nicolas Puillandre[3], Maria Vittoria Modica[1,8], Philippe Bouchet[3], Marco Oliverio[1]

[1] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Viale dell'Università 32, Rome 00185, Italy.
[2] Via delle ville, 79, 55013 Lammari, Lucca, Italy, luigiromani78@gmail.com.
[3] Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 51, 75005 Paris, France.
[4] Tartamare, Via Donato Bramante, 83, 58100 Marina di Grosseto (GR), Italy.
[5] Severtsov Institute of Ecology and Evolution, Russian Ac. Sci. Moscow, Russia.
[6] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution.
[7] Department of Marine Animal Conservation and Public Engagement, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy.
[8] Department of BEOM, Stazione Zoologica Anton Dohrn, Via Francesco Caracciolo, 80121 Naples, Italy.

Aim

With two million species described, it is still unclear how far we have gone in the inventory of Earth diversity. The number of existing species is estimated around 10–100 mln, with large uncertainty due to understudied phyla. Since the application of molecular biology in systematics, it became possible to detect species that could not be discovered by traditional morphological means. The impact of this "cryptic diversity" on global estimates has been investigated only in a few recent studies on insects. By analysing literature data, these studies hypothesize that the number of insect species could be 3 to 6 times larger than expected. We propose a different empirical approach for weighting the impact of cryptic species focusing on the class Gastropoda.

Methods

A large sampling dataset of over 5000 gastropods collected in Corsica during the CORSICABENTHOS expedition, which received both morphological and molecular identification, was analysed to measure the Cryptic Diversity ratio (CD-ratio), i.e. the number of expected cryptic species per morphospecies in a given taxon. Results from this method were compared with CD-ratio estimates obtained by scrutiny of literature on species delimitation.

Results

This large-scale barcoding project disclosed previously unrecognized cryptic diversity, while also establishing the taxonomic status of debated species. The resulting CD-ratio is >1, yet lower than the one extrapolated from literature data.

Main conclusion

Despite the Mediterranean being a very well-studied marine bioregion, our analyses confirm that cryptic diversity does impact global marine biodiversity estimates. However, the magnitude of such impact cannot be adequately inferred from literature meta-analyses. Finally, CD-ratio has surely a large geographic and taxonomic variance, making extrapolations to a global scale problematic. Revision of neglected taxa by Integrative Taxonomy approaches remains needed to understand the real impact of cryptic diversity on the global diversity estimates.

Conservation

MicroRNA-mediated environmental adaptation in microalgae: from physiological responses to the identification of novel biomarkers.

Gabriele De Falco[1], Luca Ambrosino[2], Chiara Lauritano[1], Sandro Banfi[3,4], Sabrina Carrella[1]

[1] Ecosustainable Marine Biotechnology Department, Stazione Zoologica Anton Dohrn, Naples, Italy.
[2] Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy.
[3] Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy.
[4] Medical Genetics, Department of Precision Medicine, University of Campania "L. Vanvitelli", Naples, Italy.

Aim

MicroRNAs (miRNAs) are small non-coding RNAs that can regulate gene expression post-transcriptionally by interacting with mRNAs. They are fine tune modulators that have a pleiotropic effect, interacting with different targets. Their expression is reported to vary according to external stimuli, triggering cellular responses and metabolic adjustments, becoming important modulators in the adaptation to environmental conditions or stress. Remarkably, a few or even a single miRNA can be used to diagnose stress response, which makes them particularly attractive as potential indicators of population-level stress responses.

Methods

We selected the microalga *Phaeodactylum tricornutum* in order to observe the differential expression of miRNAs under three different stress conditions: low salinity, nitrogen deprivation, and metals contamination (Cd + Pb). We selected these conditions because could represent some environmental changes that are important to be monitored. Specifically, the total RNA of the microalga under all the conditions and control condition has been extracted and then sequenced by small-seq TruSeq (Illumina) in order to construct small RNA libraries.

Results

We will identify miRNAs via mapping on miRbase to find the already known ones and the possible conserved ones (by also using miREvo). The mapping of unknown sequences on the *P. tricornutum* genome will allow the discovery of novel miRNAs via the use of bioinformatic tools (such as miRDeep2). Furthermore, it will be analysed the Differential Expression of the miRNAs (DEMs) in each condition and possible direct targets will be predicted (via the use of psRNATarget, miRanda, psRobot) and validated.

Main conclusion

This study will be helpful in the elucidation of the molecular adaptation of diatoms to different environmental stresses and in the identification of novel molecular biomarkers.

Conservation

Hidden in the ocean: the importance of detecting hybridisation in pelagic and deep-water fishes

Carlo Pecoraro[1], Chiara Papetti[2,3,4,5], Carmelo Fruciano[5,6]

[1] Physalia-courses, Berlin, Germany.
[2] Department of Biology, University of Padova – via U. Bassi, 58/B – 35121 Padova, Italy.
[3] Department of Integrative Marine Ecology, Zoological Station Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.
[4] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa) – 00196 Roma, Italy.
[5] National Biodiversity Future Center, Palermo, Italy.
[6] Institute for Marine Biological Resources and Biotechnology (CNR-IRBIM), National Research Council. Via S. Raineri – 4 98122 Messina, Italy.

Abstract

The intrigue of fish hybridisation holds profound implications for both the fundamental principles of biology and practical conservation and management efforts. Despite evidence of its pervasiveness, the phenomenon of hybridisation in fish is not uniformly studied across species and environments. We unveil a consistent underestimation of hybridisation in these groups, shedding light on a critical blind spot. We note how natural hybridisation in pelagic and deep-sea fish has been rarely reported. For this reason, we carry out an analysis using both standard and phylogenetic comparative methods. Our results suggest a lack of evidence for the idea that pelagic and deep-sea fish are inherently less prone to hybridise. Likely, hybridisation and introgression are systematically underestimated in these groups. In light of this, we discuss why underestimation of hybridisation is problematic, and what it may be done to ameliorate the situation.

We propose scalable and cost-effective prioritisation, sampling, and analysis strategies, to ease existing biases in assessing the impact of hybridisation among pelagic and deep-sea species and to ultimately improve the management and conservation– as well as basic biological knowledge – of these important species.

Our work calls for a shift in perception and action, inviting a holistic approach to studying and safeguarding these vital species.

Conservation

Teach a man to fish and we may increase seahorses' protection: evidence from citizen science and spatial ecology in the Italian *Hippocampus* species

Raffaele Panzuto[1,2], Luciano Bosso[3], Rosario Balestrieri[1], Sonia Smeraldo[4], Maria Luisa Chiusano[3], Francesca Raffini[5], Luigi Musco[6], Claudia Gili[2]

[1] Marine Animal Conservation and Public Engagement, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy
[2] Department of Ecological and Biological Sciences, University of Tuscia, 01100, Viterbo, Italy
[3] Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy
[4] Experimental Zooprophylactic Institute of Mezzogiorno, Via della Salute, 2, Portici, 80055 Naples, Italy
[5] Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy
[6] Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy

Abstract

Citizen science and spatial ecology analyses can inform species distributions, habitat preferences, and threats in elusive and endangered species such as seahorses. Through a dedicated citizen science survey submitted to the Italian diving points, we collected 115 presence records of the two seahorses occurring in this country, *Hippocampus hippocampus* and *H. guttulatus*. This data was then used to map the ecological features of these poorly known species and quantify the effects of current human activities on their habitat suitability through geographic information systems and ecological species distribution modelling. Our results indicated a quite continuous suitable area along the Italian coasts with a major gap in the central Adriatic Sea (Emilia-Romagna and Marche) in both seahorses. They co-occurred in most of their distribution, particularly in the central and southern Tyrrhenian coasts, and their ecological niches resulted to be significantly similar but not equivalent. The least-cost paths of both species were concentrated in the Southern Italy (Apulia, Calabria, and Sicily), suggesting that more data is needed to improve the spatial resolution of the available information, especially in the Northern and Central Italy. Human activities affected 35% and 41% of the *H. hippocampus'* and *H. guttulatus'* habitat suitability, respectively, while only 31% and 34% of their potential distribution, respectively, is protected by the existing system of conservation areas in Italy, in line with the seahorses' worldwide average. In particular, the central Adriatic Sea represents a critical area where the occurrence of these seahorses is lower and the anthropic impact is higher. Considering all the Italian regions, fishing effort is the main human activity impacting both the seahorses. Our findings directly assist more efficient conservation actions. We encourage the application and interaction of citizen science and spatial ecology analyses to facilitate the assessment and sustainable management of elusive organisms.

Conservation

Posidonia oceanica adaptative responses to ocean acidification

Katiuscia Petrosillo[1,2], **Maria Cristina Gambi**[1], **Cinzia De Vittor**[1], **Valentina Esposito**[1]

[1] National Institute of Oceanography and Applied Geophysics – OGS, Trieste, Italy
[2] University of Palermo, Italy.

Aim

The increase of anthropogenic CO_2 in the atmosphere is one of the main drivers of global change in the oceans, leading to an increase in temperature and ocean acidification (OA). Areas naturally enriched in CO_2, such as volcanic vents, have been used to study the effects of future acidification on marine ecosystems. Seagrasses are important habitat formers, and *Posidonia oceanica* is the endemic and most widespread species in the Mediterranean that can also colonize high pCO_2/low pH habitats. Here we summarize the studies conducted in hydrothermal vents on *P. oceanica* and its associated fauna to shed light on adaptive responses to OA.

Methods

A bibliographic review was conducted for a total of 18 selected references.

Results

Posidonia oceanica responds to high pCO_2 with an increase in primary production and shoot density as well as in leaf growth, height and thickness. However, under some extreme conditions in the Panarea vents, dwarf shoots were described with a biomass up to 75 % lower than normal shoots. Alteration in stable isotope signature and higher C/N ratio were measured in the leaves, possibly explaining the high grazing pressure by the herbivore fish *Sarpa salpa*. A simplified epiphytic community with a strong decrease of all calcareous forms occurred in various systems, while the motile fauna showed higher abundances and comparable biodiversity respect to normal pH conditions. Finally, *P. oceanica* meadows are able to modify pH up to 0.5 pH units daily through photosynthetic activity and community metabolism, providing a chemical refuge for associated fauna.

Main conclusions

The summarized adaptive responses to OA in Posidonia have far-reaching direct and cascading effects on associated communities and ecosystem services. Since not all the responses were univocal and predictable (e.g. dwarf shoots), further studies on OA and synergistic effects of other stressors on this habitat are essential to unveil the mechanisms of its survival.

Conservation

Analysing life-history and ecological traits to investigate the response to protection of rocky-reef fish community in a Mediterranean Marine Protected Area

Federica Poli[1], Simone Bava[1], Elena Desiderà[1], Davide Di Blasi[1], Paolo Guidetti[1]

[1] Department of Integrative Marine Ecology (EMI), Genoa Marine Centre (GMC), Stazione Zoologica Anton Dohrn–National Institute of Marine Biology, Ecology and Biotechnology, Villa del Principe, Piazza del Principe 4, 16126 Genoa, Italy.

Aim

Marine Protected Areas (MPAs) provide protection populations and ecosystems, while allowing for a regulated resources' use, e.g., fishing. The time scale required by biological systems to respond to protection measures (i.e., the reserve effect) poses a big challenge in the MPAs management. Assessing early responses to protection is therefore crucial to adjust MPAs design and maximize their performance over time. The extent and the timing of populations' recovery is influenced by species life histories and ecological traits. Here, we evaluated the reserve effect at the Cinque Terre MPA (Italy) on the whole rocky reef fish community also by grouping species according to their traits' similarity to better interpret their recovery trajectories.

Methods

We analysed biomass data of the rocky-reef fish community outside and within the three protection levels of the Cinque Terre MPA. We then built a matrix of species life history and ecological traits potentially influencing populations' response to protection (i.e., planktonic phase duration; commercial value; mobility etc.). Finally, biomass data trends have been re-analysed by pooling species according to traits' correlations.

Results

Traits' analysis showed that species clustered into three groups characterized by differences in mainly four traits: prey guild, planktonic phase duration, gregariousness, and commercial value. Species feeding on high trophic levels, having long planktonic larval development, intermediate level of gregariousness and high commercial value showed a significant response to protection, while other groups demonstrated a limited reserve effect or none.

Main conclusion

Traits' analysis allows to disentangle the complex ecological interactions that could mask populations' recovery. Grouping species based on their traits' similarity could help in assessing early responses to protection, whilst allowing to adjust MPA measures, maximise their effectiveness and simplify their management. Overall, traits' analysis has the potential to become a crucial tool to study fish population responses to MPA protection.

Conservation

Adaptive mechanisms to Ocean Acidification of different limpet species from the CO_2 vent systems of Ischia Island (Italy)

Silvia Giorgia Signorini[1], Marco Munari[2], Fabio Crocetta[3], Ilaria D'Aniello[2], Lara Nigro[1], Camilla Della Torre[1]

[1] Department of Biosciences, University of Milan, Milan, Italy - silvia.signorini@unimi.it; lara.nigro@unimi.it; camilla.dellatorre@unimi.it.
[2] Department of Biology, Stazione Idrobiologica Umberto d'Ancona, University of Padova, Chioggia (Venice), Italy - marco.munari.1@unipd.it; ilariadaniello97@gmail.com.
[3] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy - fabio.crocetta@szn.it.

Aim

From the beginning of the industrial era, ocean has globally absorbed more than 500 billion tons of CO_2. As a major consequence of such event, seawater has become more acidic and has undergone major changes in its carbonate chemistry, leading to the phenomenon commonly known as Ocean Acidification (OA).

Species of the genus *Patella* Linnaeus, 1758 are shelled gastropods mostly living in the intertidal area. Despite of the effects that a decreased pH has on calcifying organisms, these taxa thrive also under naturally acidified conditions. We hereby investigated the biochemical and molecular mechanisms that allow survival and adaptation of these species at the CO_2 vents of the Castello Aragonese (Ischia Island, Tyrrhenian Sea).

Methods

Individuals of *Patella caerulea*, *P. rustica*, and *P. ulyssiponensis* were collected in February 2023 along a pH gradient on the north site of the Castello Aragonese vent system, as well as at San Pietro, located at a distance of 4 km from the vent and characterized by ambient pH. Morphometric characteristics, oxidative stress, energy metabolism, and neurotoxicity endpoints were investigated in the three species, whereas physiological traits and untargeted metabolomics in *P. caerulea* only.

Results

An induction of some oxidative stress endpoints was observed in all the species. A modulation of the acetylcholinesterase activity was found in P. *ulyssiponensis* and *P. rustica* as a function of pH, while glycogen content was higher in *P. cerulea* and *P. rustica* from the lower pH site with respect to the ambient site. Metabolomics revealed a significative modulation of different metabolites, mainly linked to carnitine metabolism in specimens living in acidified conditions.

Main conclusion

These preliminary results suggest that OA might entail an oxidative stress condition in organisms and that a metabolic cost underpins tolerance to OA.

Conservation

Investigating the thermal indicators linked to flowering and fruiting of the seagrass *Posidonia oceanica* and estimating the uncertainties due to local factors

Patrizia Stipcich[1], Gabriella La Manna[1,2], Giulia Ceccherelli[1]

[1] Dipartimento di Scienze Chimiche, Fisiche, Matematiche e Naturali, Università di Sassari, Italy; pstipcich@uniss.it, cecche@uniss.it
[2] MareTerra Onlus – Environmental Research and Conservation, Alghero, Italy; gabriella.lamanna@gmail.com

Aim

The increase in sea surface temperature (SST) and in the occurrence of extreme climatic events are affecting marine ecosystems at multiple levels. To overcome the continuous environmental changes, seagrasses have developed adaptative strategies such as changes in patterns of sexual reproduction, distribution shifts, and morphological changes that allow to maintain the provision of goods and services. Past studies highlighted that flowering and fruiting events of the endemic seagrass *Posidonia oceanica* have been increased in the past decades in the Mediterranean Sea, and this study aimed at investigating the role of the high temperature on these phenomena.

Methods

To shed a light on the thermal conditions that might trigger flowering, the relation between several thermal descriptors and *P. oceanica* flowering and fruiting variables were tested in this study, gaining data from five areas in Sardinia (Italy) in Autumn 2022 and Winter 2023.

Results

Results suggested that mean summer SST was positively associated to inflorescence density, presence of living fruits and fruit size; living fruits and fruit size were also affected positively by the maximum SST within the 3 months before sampling (November-February). Both flowering and fruiting were negatively affected by the maximum SST within one month before inflorescence observation and mean winter SST, respectively.

Main conclusion

Overall, this study suggested that *P. oceanica* reproductive effort depends on the temporal context in which the anomaly occurs and its duration. However, the high variability between meadows of the same area indicates the important role of local factors in affecting flowering that still need to be highlighted.

Conservation

Whole mitochondrial genome sequencing provides new insights into the phylogeography of loggerhead turtles (*Caretta caretta*) in the Mediterranean Sea

Livia Tolve[1], Alessio Iannucci[1,2], Luisa Garofalo[3], Andrea Ninni[4,5], Andrea Capobianco Dondona[6], Ilaria Ceciarini[7], Cristiano Cocumelli[3], Alessandra De Lucia[8], Mattia Falconi[4], Angela Formia[1,9], Federico Iacovelli[4], Cecilia Mancusi[7,10], Erica Marchiori[11], Letizia Marsili[7], Toni Mingozzi[12], Stefano Nannarelli[8], Chiara Natali[1], Giuliana Terracciano[13], Marco A. L. Zuffi[14], Andrea Novelletto[5], Claudio Ciofi[1]

[1] Department of Biology, University of Florence, 50019 Sesto Fiorentino (FI), Italy.
[2] National Biodiversity Future Center, 90133 Palermo, Italy.
[3] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", 00178 Rome, Italy.
[4] Department of Biology, Tor Vergata University of Rome, 00133 Rome, Italy.
[5] PhD Program in Evolutionary Biology and Ecology, Tor Vergata University of Rome, 00133 Rome, Italy.
[6] Farm4Trade, 66041 Atessa (CH), Italy.
[7] Department of Physical Sciences, Earth and Environment, University of Siena, 53100 Siena, Italy.
[8] Hydrosphera onlus, Rome, Italy.
[9] African Aquatic Conservation Fund, Chilmark, MA 02535, USA.
[10] Agenzia Regionale per la Protezione Ambientale Toscana (ARPAT), 57125 Livorno, Italy.
[11] Department of Comparative Biomedicine and Food Science, University of Padua, Agripolis, 35020 Legnaro (PD), Italy.
[12] Department of Biology, Ecology and Earth Sciences, University of Calabria, 87030 Rende (CS), Italy.
[13] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", 56123 Pisa, Italy.
[14] Natural History Museum, University of Pisa, 56011 Calci (PI), Italy.

Aim

For decades, phylogeographic studies of marine turtles have relied on comparisons of partial mitochondrial DNA (mtDNA) control region sequences. The limited resolution of this marker due to common haplotypes being shared across populations, has hampered a comprehensive description of lineages and the assignment of turtles of unknown origin to their rookeries. Analysis of whole mtDNA provides a tool for a better characterization of matrilines. We applied a mitogenomic approach to the phylogeography of the loggerhead turtle (*Caretta caretta*), a conservation-dependent species in the Mediterranean Sea.

Methods

Mitochondrial genomes of 61 *C. caretta* individuals sampled in the central Mediterranean Sea were sequenced by synthesis at high coverage on an Illumina NovaSeq 6000 platform. Five additional loggerhead mitogenomes from the Atlantic and Pacific Oceans were also included in the alignment. We assigned mtDNA haplotypes and haplogroups. We then estimated population genetic diversity for mtDNA control region and complete mitogenome haplotypes and performed Maximum Parsimony and Bayesian phylogenetic analysis.

Results

We recovered 23 mitogenome haplotypes and 286 polymorphic sites versus 10 haplotypes and 55 polymorphic sites obtained by analysis of partial mtDNA control region sequences alone. In particular, the common mtDNA control region haplotype CCA2.1 presented 11 mitogenomic variants. Phylogenies showed that the Mediterranean/Atlantic haplogroup expanded recently with respect to the time of divergence from the sister clade - the Pacific haplogroup - and the Atlantic haplogroup.

Main conclusion

Whole mitogenome sequencing proved a powerful tool to better characterize maternal lineages of *C. caretta* and reconstruct phylogenetic relationships. Application of mitogenomic studies to a comprehensive dataset representing the main Mediterranean, Atlantic and Pacific populations will allow detailed reconstruction of worldwide dispersal and colonization patterns of loggerhead turtles.

Conservation

The Effects of Short-Term Acute Low-Light Exposure on the Coral *Pachyseris speciosa* from Singapore's Turbid Reef: a Photo-Physiological and Transcriptomic Approach

Lorenzo Massimo Toniolo[1,2], Danwei Huang[3,4], Ow Long Wei Long[3], Khaw Chee Haw Jonas[3], Chenoa Bo Kannan[3], Lionel Ng Chin Soon[3,4], Leong Wai[3], Davide Seveso[1,2], Yohan Louis[1,2], Jani Tanzil[3]

[1] Department of Earth and Environmental Sciences, University of Milano - Bicocca, Piazza della Scienza, 1, Milano 20126, Italy. l.toniolo2@campus.unimib.it.
[2] MaRHE Center (Marine Research and High Education Center), Magoodhoo Island, Faafu Atoll, Maldives. davide.seveso@unimib.it.
[3] Tropical Marine Science Institute, National University of Singapore, S2S Building, 18 Kent Ridge Road, Singapore 119227. jani.tanzil@nus.edu.sg.
[4] Department of Biological Sciences, National University of Singapore, Block S3 Level 4, 16 Science Drive 4, Singapore 117558. huangdanwei@nus.edu.sg.

Aim

Coral reefs worldwide are currently under multiple threats due to increase sea surface temperature and other abiotic, biotic, and anthropogenic factors. In particular, Singapore's reefs suffer from high sedimentation and low light penetration as a result of extensive land reclamation coastal development since the 1960s. Underwater visibility in the Singapore Strait has reduced from >10m in the early 1960s to ~2m today, with average daily photosynthetically active radiation (PAR) levels at ~6m depths reaching <50 μmol m^{-2} s^{-1}. The current study aims to investigate the effects of extreme low-light stress on the photo-physiology of the common Singapore's species *Pachyseris speciosa*.

Methods

Four different colonies of *P. speciosa* were sampled from Kusu Island, a reef site in the Singapore Strait, and 16–18 fragments were obtained from each colony. These fragments were subjected to three different light treatments for 4 weeks; 1) Control – normal light conditions (PAR = 16.4 ± 38.5 μmol m^{-2} s^{-1}); 2) Intermittent – 5 days of normal light and 2 days of total darkness; 3) Dark – always fully shaded in total darkness (PAR = 0.8 ± 8.0 μmol m^{-2} s^{-1}). Photo-physiological (zooxanthellae density, chlorophyll concentration, and PAM fluorescence) and transcriptomic data were recorded from every fragment at the beginning and at the end of the experiment. In addition, colour monitoring of all fragments was done twice a week.

Results

After a 4-week experiment, all 68 fragments of *P. speciosa* survived. Chlorophyll concentration, zooxanthellae density, health and MQY showed a significant decrease in the shaded treatment. Respectively, they decreased by 67% $p<0.01$, 71% $p<0.01$, 58% $p<0.01$, 22% ($p<0.01$).

Main conclusion

Overall, our findings suggest that *P. speciosa* is very robust to low-light stress and thereby a good candidate for use in restoration in low light environments such as those found at nearshore reefs or at deeper reef zones around Singapore.

Macroevolution

Chairs: **Maria Vittoria Modica** & **Sergio Stefanni**

This theme focuses on major evolutionary patterns and processes affecting the diversity of marine clades, above the species level, including phenotypic evolutionary trends over extended timescales.

Macroevolution

The ERC HYPERDIVERSE project: untangling drivers of hyperdiversification in neogastropods

Nicolas Puillandre[1]

[1] Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France.

Aim

Explaining why some taxa are more diversified than their closest relatives remains one of the major challenges in evolutionary biology. However, much of Earth's biodiversity is concentrated in few diverse non-vertebrate animal lineages, whose analysis of diversification dynamics is hampered by the lack of empirical data. The ERC HYPERDIVERSE project aims to understand the processes behind the evolutionary success of neogastropods, a hyperdiverse group of venomous marine molluscs.

Methods

The first objective of the project is to clarify the systematics of the group, by applying integrative taxonomy to delimit species and speed up their description and by using an exon-capture approach to reconstruct a genus-level phylogeny. The second objective is to identify in the genomes and transcriptomes of selected species the toxins that they use to subdue their prey. By relying both on the reconstructed phylogeny and the estimated toxin diversity, we will then evaluate if the diversity of the neogastropods, or subgroups within neogastropods, is linked to the type and quantity of toxins they produce.

Results

Hundreds of new species have been identified so far, and the phylogeny obtained as part of the project includes around 1,000 genera, sequenced for 1,100 exons, and most nodes are highly supported, revealing some unexpected relationships. The first chromosome-level genomes confirmed the presence of a whole genome duplication for the neogastropods, potentially linked to the apparition of new toxin-related genes, that might partly explain their evolutionary success.

Main conclusion

The last two years of the project will be devoted to the analysis of the macroevolutionary patterns, relying both on the phylogeny to estimate diversification rate variation and on estimated toxin diversity to correlate it to the species diversity among lineages. But what is already clear from our results is the importance of a good knowledge of the species diversity and phylogenetic relationships, essential when tackling macroevolutionary questions.

Macroevolution

"The molecular basis of circadian rhythms in echinoderm larvae"

Tanya Alessandro[1], Rossella Annunziata[1]

[1] Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, 80121, Naples, Italy;
tanya.alessandro@szn.it; rossella.annunziata@szn.it.

Aim

Environmental periodicities influence physiology and behavior of most living organisms on Earth, therefore sophisticated timing mechanisms have evolved to adapt to these periodicities. Molecular regulation of circadian rhythms has been described in animals as well as in plants, fungi and bacteria while information on non-chordate deuterostomes such as echinoderms is scarce and a detailed study of echinoderm larval transcriptomic and physiological activity during the light-dark cycle is missing.

Methods

To explore the evolutionary conservation of the canonical circadian clock genes, we exploited the available genomic and transcriptomic databases of species belonging to the Ambulacraria clade. Phylogenetic analyses are ongoing and will shed light on their evolutionary conservation in deuterostomes. The transcriptomes of the sea urchin *Paracentrotus lividus* and the sea star *Patiria miniata* larvae entrained in 12L:12D will be examined by RNA-seq and Real Time-PCR. The spatial expression of the putative echinoderm circadian clock components during the development of sea urchin and sea star embryos up to the larval stage will be assessed through ISH and their involvement in the regulation of circadian rhythms will be tested through functional studies, using gene perturbation tools.

Results

The majority of the considered circadian clock genes has been identified in the selected species. Interestingly, the well-conserved clock component *Period* was not found in none of the analysed echinoderm and hemichordates species, suggesting its loss in Ambulacraria during deuterostome evolution.

Main conclusion

Our results highlight that the Ambulacraria core clock architecture might be organized differently from the animal circadian clocks so far investigated in metazoans. This study offers perspectives to elucidate timekeeping mechanisms in non-chordate deuterostomes, opening the way to introduce them as experimental system in the Chronobiology field.

Macroevolution

Contrasting patterns of conservation and divergence of *Hox* genes in molluscs

Giuseppe Emanuele De Pasquale[1], **Jacopo Martelossi**[1], **Filippo Nicolini**[1,2], **Liliana Milani**[1], **Fabrizio Ghiselli**[1]

[1] Department of Biological Geological and Environmental Science, University of Bologna, via Selmi 3, 40126 Bologna, Italy.
[2] Fano Marine Center, Department of Biological, Geological and Environmental Sciences, University of Bologna, Viale Adriatico 1/n, 61032 Fano, Italy.

Aim

Differently from what observed in most Metazoa, *Hox* gene expression timing and localization during mollusc embryo development cannot be easily framed in a collinearity perspective. The aim of this study is to investigate the organization, diversity, and evolution of the *Hox* gene cluster in molluscs, focusing on the classes Bivalvia, Cephalopoda, and Gastropoda.

Methods

A broad characterization of the *Hox* gene cluster was performed by analysing genome assemblies of 35 species, representing 18 distinct families within the phylum Mollusca. Molecular evolution analyses were performed to estimate the selection pressures acting on *Hox* gene sequences. Additionally, the amino acid composition was explored within the Hox domain to identify specific residues characterizing each gene family.

Results

We found a coherent organization of *Hox* genes across the three classes, with a consistent genomic arrangement, following the order *hox1-5*, *lox5*, *antp*, *lox4*, *lox2*, *post2*, and *post1*, with the latter gene always located on the complementary strand. We found intriguing patterns in genic and intergenic distances within the *Hox* cluster, which varied depending on the gene family. The molecular evolution analyses provided evidence of negative/purifying selection acting on some sites within the *Hox* gene sequences and episodic diversifying selection in some tree branches.

Main conclusion

This study provided insights into the organization and diversity of *Hox* genes in the phylum Mollusca. The coherent genomic arrangement and conservation of Hox sequences, as well as the evidence of episodic diversifying selection across gene families, highlight the pivotal role of Hox genes in body plan development, but also the potential for contributing to the diversification of molluscan lineages. These findings open exciting perspectives to unravel the mechanisms that drive the expression and functional dynamics of *Hox* genes.

Macroevolution

Shell evolution in Velutinoidea: a phylogenetic approach

Giulia Fassio[1,3], Giacomo Chiappa[1], Elisa Nocella[1,2], Thomas Lemarcis[3], Tsuyoshi Takano[4,5], Yasunori Kano[4], Nicolas Puillandre[3], Philippe Bouchet[3], Nancy Treneman[6], Manuel António E. Malaquias[7], Maria Vittoria Modica[1,2,3], Marco Oliverio[1,3]

[1] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Italy.
[2] Department of BEOM, Stazione Zoologica Anton Dohrn, Naples, Italy.
[3] Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 51, 75005 Paris, France.
[4] Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan.
[5] Meguro Parasitological Museum, 4-1-1 Shimomeguro, Meguro, Tokyo 153-0064, Japan.
[6] Oregon Institute of Marine Biology, POB 5389, 63466 Boat Basin Road, Charleston, OR 97420, USA.
[7] Department of Natural History, University Museum, University of Bergen, PB7800 5020 Bergen, Norway.

Aim

The caenogastropod superfamily Velutinoidea comprises >460 accepted extant species, currently classified into three families: Triviidae (293 species), Eratoidae (69 species) and Velutinidae (>100 species). This group of marine snails is almost exclusively associated to tunicates for feeding and reproduction, with a remarkable variation in shell morphology: the calcified and thick shell of triviids and eratoids is traditionally considered as plesiomorphic in the superfamily, while velutinids show a trend towards a fragile shell, often internal, frequently decalcified. We aim at studying with a phylogenetic approach on a taxonomically wide, multi-locus dataset the evolution of shell shape in the superfamily Velutinoidea.

Methods

The multi-locus and single-gene datasets were assembled with sequences of mitochondrial and nuclear markers for 153 species belonging to eratoids, triviids, and velutinids. The partitions were analysed by Bayesian inference and Maximum Likelihood approach and compared to produce a robust phylogenetic hypothesis.

Results

Our results suggested velutinids as a polyphyletic group (confirmed independently by nuclear and mitochondrial markers), with relevant implications for multiple shell reduction events that may have occurred at least three times in the history of Velutinoidea.

Main conclusion

Contrary to what it is thought to be the most parsimonious route in shelled mollusc evolution (from an external strong shell to a vestigial internal one), Velutinoidea showed an unexpected great plasticity in shell evolution (with multiple shifts between these two states) possibly facilitated by an easiness in switching the genetic pathways regulating it. Several environmental conditions may have positively selected lineages with vestigial shells, like deep, cold and/or acid water.

Macroevolution

Evolution of the small RNA pathways in animals and the loss of endo-siRNAs among Lophotrochozoa

Alessandro Formaggioni[1], Gianmarco Cavalli[1], Mayuko Hamada[2], Tatsuya Sakamoto[2], Federico Plazzi[1], Marco Passamonti[1]

[1] University of Bologna, Department of Biological, Geological and Environmental Sciences.
[2] University of Okayama.

Abstract

In animals, three main RNA interference mechanisms have been described so far, which respectively maturate three types of small noncoding RNAs (sncRNAs): miRNAs, piRNAs and endo-siRNAs. The diversification of these mechanisms is deeply linked with the evolution of the Argonaute gene superfamily since each type of sncRNA is loaded by a specific Argonaute homolog protein. Moreover, other protein families play pivotal roles in the maturation of sncRNAs, like the DICER ribonuclease family, whose DICER1 and DICER2 paralogs maturate respectively miRNAs and endo-siRNAs. Among Metazoa, the distribution of these families has been only studied in major groups, and there are very few data for clades like Lophotrochozoa. Thus, we here inferred the evolutionary history of the animal Argonaute and DICER families including 43 lophotrochozoan species. Phylogenetic analyses along with newly sequenced sncRNA libraries depicted a loss of the endo-siRNA pathway along the Lophotrochozoa evolution, with the absence of DICER2 in Nematoda and Polyzoa, and with the absence of DICER2 and the Argonaute homolog in the rest of Trochozoa phyla. On the contrary, early diverging phyla, Platyhelminthes and Syndermata, showed a complete endo-siRNA pathway. On the other hand, miRNAs were revealed the most conserved and ubiquitous mechanism of the metazoan RNA interference machinery, confirming their pivotal role in animal cell regulation.

Macroevolution

Novel insights on *Ciona robusta* gut physiology and evolution

Marco Gerdol[1], Rita Marino[2], Michelina Plateroti[3], Annamaria Locascio[2], Maria Sirakov[2]

[1] Department of Life Sciences, Università degli Studi di Trieste, via Licio Giorgieri 5, 34127 Trieste, Italy.
[2] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy.
[3] University of Strasbourg, Inserm, UMR-S1113 / IRFAC, FHU ARRIMAGE, FMTS, 67200 Strasbourg, France.

Abstract

This work proposes to investigate the anatomy and molecular features of the gastrointestinal tract (*i.e.* gut) of the ascidian *C. robusta*, focusing on the adult stage. This protochordate shares several functional and morphogenetic traits with vertebrate models. Worthily, its non-duplicated and fully sequenced genome is available. Several similarities of Ciona and mouse gut have already been shown but the detailed molecular mechanisms of this regionalization together with its associated functionalities are still poorly understood.

We took a good look at the tissue architecture along the antero-posterior axis of *Ciona* gut. Then we performed an RNAseq analysis of the stomach and two different intestinal tracts to determine and to reconstitute the signalling pathways and molecular components expressed along the gastrointestinal tract. In particular, we used the well-known mouse *Mus musculus* as vertebrate model in order to compare and shed light on gut evolutionary and/or functional aspects.

The final goal of this research is to highlight some aspects of Ciona gut evolution and physiology. Our results can fit in a frame of evolutionary studies aimed to identify the molecular players acting to establish cell types distribution and their function in the different tissues. Such investigations can be of pivotal importance in defining major aspects of animal evolution.

Macroevolution

Signatures of extreme longevity: a perspective from bivalve molecular evolution

Mariangela Iannello[1], Giobbe Forni[1], Giovanni Piccinini, Ran Xu[1], Jacopo Martelossi[1], Fabrizio Ghiselli[1], Liliana Milani[1]

[1] Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy.

Aim

Longevity is a complex and poorly characterized trait: we do know that some species can live longer than others, but the molecular players responsible for the multiple independent occurrences of the extended longevity phenotype are largely unknown. In this context, bivalve molluscs can provide novel perspectives: the class Bivalvia shows indeed the highest lifespan disparity within Metazoa, ranging from 1 to 500+ years, and includes the longest-lived non-colonial animal species known so far, the clam *Arctica islandica*. Bivalves therefore represent important resources to provide insights into the evolution of extended longevity.

Methods

In this work, we leveraged transcriptomic resources spanning thirty bivalve species and we investigated genes with signature of convergent molecular evolution in bivalves showing extreme longevity. We analyzed, for the first time for such topic, networks of protein interactions among such genes, to have a more comprehensive view of the interplay of genes and pathways co-involved in the extended longevity phenotype.

Results

A large majority of genes showing convergent evolution in long-lived bivalves constitute a large, continuous network of connections. Such network is enriched for factors with experimental support for a role in longevity in other animal species. The network highlights that an integration of different genes and pathways is required for the extended longevity phenotype, and genes involved in cell proliferation control, translational machinery, and response to hypoxia seem to have a central role in lifespan extension.

Main conclusions

Our results suggest that the mechanisms underlying extended longevity are, at least partially, similar across metazoans: while some genes in the network have experimental support of a role in longevity in model species, and they are likely involved in longevity in bivalves, other genes in the network may represent new possible candidates with a role in extending lifespan, both in bivalves and other animals.

Macroevolution

Genomic traits of the water-to-land transition in brachyurans: the terrestrial crab *Chiromantes haematocheir* as a model species

Alessio Iannucci[1,2], Roberto Biello[3], Andrea Benazzo[3], Christine L. Y. Chen[4], Ka Hei Ng[4], Giovanni Bacci[1], Duccio Cavalieri[1], Claudio Ciofi[1], Alessio Mengoni[1], Niccolò Meriggi[1], Stefano Cannicci[1,2,4], Sara Fratini[1,2]

[1] Department of Biology, University of Florence, Sesto Fiorentino, Italy.
[2] National Biodiversity Future Center, Palermo, Italy.
[3] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy.
[4] The Swire Institute of Marine Science, The University of Hong Kong, Hong Kong, Hong Kong SAR.

Aim

The transition of aquatic species to terrestrial environments occurred repeatedly in all animal groups. Aquatic and terrestrial habitats are characterized by extremely different conditions and nearly every life history trait was affected during the conquest of dry land. Genomics has proven an effective tool for the study of terrestrialization thanks in particular to the discovery of gene groups involved in the water-to-land transition of several taxa. Although semi-terrestrial and terrestrial brachyuran crabs are a key group to understand the metabolic mechanisms involved in the transition from water to land, no genomic studies have so far considered patterns of land colonization in this taxon. True crabs (Decapoda; Brachyura) have been particularly successful in evolving semi-terrestrial and terrestrial life habits and count more than 20 phylogenetically unrelated families of Brachyura that conquered the land. We used genomic and transcriptomic tools to study the molecular traits involved in the terrestrialization process of brachyurans using the terrestrial crab *Chiromantes haematocheir* as a model species.

Methods

We sequenced and assembled a draft genome of *Chiromantes haematocheir* using both long- and short-reads sequencing approaches. We then performed transcriptome sequencing on *C. haematocheir* individuals kept in water or air-exposed. Expansion and contraction of differentially expressed genes were then investigated in malacostracan species using an annotated genome.

Results and Conclusions

The assembled genome of *C. haematocheir* had a size of 3.1Gb, an N50 of 200kb and a busco score of ~93%. Transcriptome analyses revealed differential expression in *C. haematocheir* kept in water versus those individuals exposed to air. Differentially expressed genes included those involved in the regulation of inducible factors, which play a role in sensing molecular oxygen. Analysis of expansion and contraction of these genes among malacostracan species is in progress and will be used to better understand patterns of genomic adaptation that led marine crabs to colonize terrestrial ecosystems.

Macroevolution

From tunicates to vertebrates, evolution of Nitric oxide signaling

Annamaria Locascio[1], Quirino Attilio Vassalli[1], Immacolata Castellano[1], Anna Palumbo[1]

[1] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy.

Aim

Nitric oxide (NO) is a key signalling molecule in almost all organisms and is active in a variety of physiological and pathological processes.

In non-vertebrate chordates only one *Nos* gene is present, whereas in mammals three *Nos* gene copies have been identified, by extending studies to a non- vertebrate chordate, the ascidia *Ciona robusta*, we investigated the evolution among chordates of Nos/NO distribution and biological function.

Results

NOS/NO localization patterns during the different stages of *Ciona* larval development evidenced a highly dynamic localization profile that perfectly matches with the central role played by NO from the first phase of settlement induction to the next control of swimming behavior, adhesion to substrate, and progressive tissue resorption of metamorphosis. This specific and temporally controlled pattern of NO starts in the most anterior sensory structures of the early larva and progressively moves towards the caudal portion as larval development and metamorphosis proceeds.

Conclusions

Our comparative analysis of the function of Nos/NO among chordates from cephalochordates to tunicates, teleost fishes and amphibians provided a comprehensive set of data showing a high degree of conservation of No functions and adding new elements to our understanding of Nos evolution. Whereas in mammals NO acts mainly as neurotransmitter, vasodilator, and immune response mediator, in marine invertebrates the gas is involved in a variety of biological processes, including defence,, neurotransmission, swimming, feeding, symbiosis and development

Macroevolution

A timescale to sea spiders' evolution (Arthropoda: Pycnogonida)

Morena Nava[1,5], Romain Sabroux[2], Sandra Álvarez-Carretero[2], Philip C. J. Donoghue[2], Gregory D. Edgecombe[3], Russel J. Garwood[3,4], Davide Pisani[2,5]

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, I-20126 Milan, Italy.
[2] Bristol Palaeobiology Group, School of Earth Sciences, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol BS8 1TQ, UK.
[3] The Natural History Museum, London SW7 5BD, UK.
[4] Department of Earth and Environmental Sciences, University of Manchester, Manchester M13 9PL, UK.
[5] Bristol Palaeobiology Group, School of Biological Sciences, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol, BS8 1TQ, UK.

Aim

Sea spiders are marine chelicerates represented by ca. 1,400 extant species distributed worldwide: from littoral to abyssal waters and from tropical to polar regions. They nest within the Arthropoda tree of life as the sister to all the other chelicerates, which makes them a key lineage to understand arthropods' early evolution.

While sea spiders are currently divided into eleven families (order Pantopoda), the fossil record testifies that their diversity was once much higher. With the aim to better understand their diversification through time, we assembled, for all we know, the most taxonomically complete molecular dataset of extant sea spiders with which, together with a reassessment of the fossil record, we inferred a Pantopoda evolutionary timeline.

Methods

The superalignment we inferred (22,384 bp), and with which we then estimated our phylogeny, consists of 13 mitochondrial protein-coding genes, the nuclear ribosomal gene 18S rRNA , and 99 UCEs of 173 species across all the extant families of Pantopoda.

Subsequently, we revisited the key fossil calibrations that had been previously used to date the Pycnogonida tree of life. After reassessing such fossil evidence, we demonstrate that some of those calibrations were based on inaccurate taxonomic hypotheses. We used our updated hard minimum and soft maximum bounds of 5 fossil specimens to constrain the node ages of Arthropoda, Chelicerata, Colossendeidae, Phoxichilidioidea, and Ascorhynchidae in our phylogeny for timetree inference with MCMCtree.

Main conclusions

According to our estimated evolutionary timeline of sea spiders, the diversification of pantopods seems to have begun between the late Ordovician and the early Silurian. Nevertheless, future consistent sampling of extant sea spiders is required to better understand their diversity and interfamilial relationships, which will in turn help to shed light into the evolutionary history of the order Pantopoda.

Macroevolution

Contrasting patterns of amino acid evolution and shared ancestry between putative sex-determining genes in bivalve molluscs

Filippo Nicolini[1,2], Fabrizio Ghiselli[1], Liliana Milani[1], Andrea Luchetti[1]

[1] Department of Biological, Geological and Environmental Science, University of Bologna, via Selmi 3, 40126 Bologna (BO), Italy.
[2] Fano Marine Center, Viale Adriatico 1/n, 61032 Fano (PU), Italy.

Aim

Bivalve molluscs hold a central role in many aspects of human economy and society, yet their sex determination system is largely overlooked. At present, the molecular mechanism by which sex is determined in bivalves is still unknown, and just few genes have been appointed as putatively involved. Nonetheless, their phylogenetic history and patterns of molecular evolution remain poorly understood.

Methods

Leveraging the growing amount of publicly available genome assemblies for bivalves, we performed an extensive survey of the *Dmrt*, *Sox* and *Fox* gene families, whose components have been frequently linked to the sex determination of bivalves. We also explored the pattern of amino acid evolution to provide additional support for their role in sex determination.

Results

Genes implicated in the male sex-determination cascade (i.e., *Dmrt1L* and *SoxH*) show high rate of amino acid evolution if compared to the entire set of bivalve orthologs. They also appear as novel acquisition of molluscs. Conversely, the gene associated to the female sex-determination cascade (i.e., *FoxL2*) does not exhibit any acceleration in the amino acid rate of evolution and is also shared with other Metazoa.

Main conclusion

In bivalves, putative male sex-determinant genes show a significantly higher rate of amino acid evolution if compared both to the putative female sex-determinant gene and to the majority of other ortholog sets. This is in line with what has been already shown for other well-studied organisms, where male-biased genes are evolving at a higher pace than female-biased and sex-unbiased genes. This work thus underlines the importance of broad comparative genomic studies to find more solid sex-determining gene candidates in species where the molecular basis of sex determination is unknown.

Macroevolution

The evolution of corallivory in the Ovulidae (Gastropoda: Cypraeoidea)

Elisa Nocella[1,2], Sofya Sergeevna Zvonareva[3], Giulia Fassio[1], Daniela Pica[4], Raimondo Villa[5], Maria Vittoria Modica[2], Marco Oliverio[1]

[1] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy.
[2] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy.
[3] Laboratory of Ecology and Morphology of Marine Invertebrates, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia.
[4] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Calabria Marine Centre, Italy.
[5] Via del Lago 2, 1-00061 Anguillara Sabazia, Italy.

Aim

Host-parasite relationships provide very useful models to study adaptive processes. We investigated the interaction between parasitic marine gastropods, the Ovulidae, and their cnidarian preys. The Ovulidae is a family of caenogastropod that feed on octocorals (Anthozoa: Octocorallia: Alcyonacea) or, to a much lesser extent, on hexacorals (Anthozoa: Hexacorallia: Antipatharia, Scleractinia) and hydrocorals (Hydrozoa: Hydroidolina: Anthoathecata: Stylasteridae). A limited information is available on the phylogenetic relationships and the degree of specificity of the predator-prey relationships within this corallivorous lineage.

Methods

To investigate ovulid/coral relationships in the context of their evolution, we generated the largest molecular dataset so far, comprising two mitochondrial (COI and 16S-rDNA) and one nuclear gene (28S-rDNA) from 524 specimens collected all over the world. To identify the coral host of the ovulid species, we integrated literature data with an empirical approach. For the former, each ovulid species present in the tree was related to the cnidarian identified in literature at the lowest possible taxonomic level. When the cnidarian host sample was available, morphological identification was conducted together with the amplification of the 16S molecular marker. Obtained sequences were matched against a reference database.

Results

The obtained molecular phylogeny of the Ovulidae revealed the existence of groups that do not completely correspond to the currently subfamily arrangement. Moreover, some of the genera as traditionally conceived were not confirmed as monophyletic. Concerning trophic ecology, our results suggest that several ovulid lineages repeatedly shifted between octocorals and hexacorals, while only the pediculariines (*Pedicularia* and allied) are associated with hydrozoans (Stylasteridae).

Main conclusion

The systematics of the ovulids should be redefined to better reflect the phylogenetic pattern emerging from the molecular analyses. Data on the coral prey of crucial species are still lacking (e.g. *Pseudocypraea* spp.), hampering a certain identification of the ancestral coral-host of Ovulidae.

Macroevolution

Fluid relationship between rates of speciation and rates of phenotypic evolution in a hyperdiverse fish clade

Gabriele Sansalone[1,2], Carmelo Fruciano[1,3]

[1] Institute for Marine Biological Resources and Biotechnology (CNR-IRBIM), National Research Council. Via S. Raineri 4 – 98122 Messina, Italy.
[2] Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 213D – 41125 Modena, Italy.
[3] Department of Biological, Geological and Environmental Sciences, University of Catania, via Androne 81 – 95124 Catania, Italy.

Aim

Why some groups of organisms achieve a spectacular richness in both species and morphological diversity whereas others do not is a question which has been engaging evolutionary biologists for many decades. Indeed, evolutionary rates often vary among clades and lineages, with fast-evolving groups accumulating new species, phenotypic diversity or both faster than slow-evolving groups. Whether rates of speciation and phenotypic evolution are correlated is, however, an open question. Classical theory focussing on adaptive evolutionary divergence suggests that these rates should be associated and has received some empirical support. At the same time, other empirical studies have failed to find an association between rates of speciation and rates of phenotypic evolution.

Methods

We move past previous efforts by using three traits – including principled projections of multivariate data. As focal clade we use wrasses, a hyperdiverse clade of marine fish comprising the families Labridae, Scaridae (parrotfish) and Odacidae. We build a new dated phylogeny of this group, and we study trait evolution and speciation using advanced geometric morphometric and statistical techniques.

Results

We find: 1. substantial rate variation within this clade for both speciation and phenotypic evolution, 2. that the relationship between speciation rates and rates of phenotypic evolution depends on the trait considered and is significant only for a specific aspect of body shape which may be interpreted in terms of genetically-driven phenotypic integration, 3. that the strength of the relationship between rates of speciation and rates of phenotypic evolution, when present, varies between fast-evolving and slow-evolving lineages.

Main conclusion

In addition to providing a deeper understanding of evolutionary patterns in wrasses, these results have broader implications for the way we address the general question of whether different rates are associated with each other.

Macroevolution

Exploring the Link Between Genetic and Morpho-Anatomical Diversity in the Swordfish's Rostra

Martina Spiga[1], Antonio Di Natale[2], Alice Ferrari[1], Fulvio Garibaldi[3], Alberto Pallavicini[4], Sara Gioele Rizzo[5], Frine Eleonora Scaglione[5], Sergio Stefanni[6], Alberto Valazza[5], Alessia Cariani[1]

[1] Department of Biological, Geological & Environmental Sciences (BiGeA), University of Bologna, 48123 Ravenna, Italy, martina.spiga5@unibo.it, alice.ferrari6@unibo.it, alessia.cariani@unibo.it.
[2] Aquastudio Research Institute, 98121 Messina, Italy adinatale@costaedutainment.it.
[3] Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, 16132 Genova, Italy fulvio.garibaldi@unige.it.
[4] Department of Life Sciences, University of Trieste, 34127 Trieste, Italy pallavic@units.it.
[5] Department of veterinary sciences, University of Turin, 10095 Grugliasco, Italy saragioele.rizzo@unito.it, frineeleonora.scaglione@unito.it, alberto.valazza@unito.it.
[6] Stazione Zoologica Anton Dohrn, 80121 Naples, Italy sergio.stefanni@szn.it.

Aim

The swordfish, *Xiphias gladius* (L. 1758), belongs to order Perciformes and represents the only living species of genus *Xiphias*. The anatomical characteristic that distinguishes adult individuals is the upper jaw, which develops enormously to form a rostrum, of a considerable size. Despite the fully preserved external morphology, Di Natale et al. (1996) defined for the first time different internal structures describe as “with canals”, characterized by bony bundles running longitudinally and separated by tissue striations of lower density and “with chambers”, characterized by multiple chambers ranging from a few to dozens of units located in the central area of the sword.

The aim of the present study is to verify the existence of an association between different internal rostrum morphologies with the occurrence of two mitochondrial haplogroups in Mediterranean specimens, as reported by several studies (Smith et al. 2015; Righi et al. 2020).

Methods

Genetic characterization was based on mitochondrial markers such as the CR (control region), NADH dehydrogenase subunit 2 (ND2), and cytochrome oxidase subunit 1 (COI); while the swordfish internal structures were inspected using x-ray or CT analysis.

Results

The haplotype networks obtained from the three mitochondrial markers support the presence of two distinct evolutionary lineages of *X. gladius* in the Mediterranean Sea, confirming the results described by various authors (see above). However, no relationship was found between the two clades and rostra morphologies when phylogenetic and genetic differentiation analyses were applied.

Main conclusion

Further alternative methodologies are currently employed to investigate the potential correlation between the genetic and morpho-anatomical diversity of the swordfish's rostra. From a genetic standpoint, we will be applying cutting-edge next-generation sequencing techniques, specifically 2bRAD. The availability of X-rays or CT scans will allow us to investigate the morpho-anatomical details of the rostra internal structure with specific programs for geometric morphometrics.

Population & Species

Chairs: **Francesca Leasi** & **Emanuela Dattolo**

This theme focuses on studies exploring the evolutionary dynamics of variations observed in populations and species across spatial and temporal scales, including, ecological, phenotypic and genetic features.

Population & Species

The Pace of Clonal Evolution in Seagrasses

Thorsten Reusch[1]

[1] GEOMAR Helmholtz-Centre for Ocean Research Kiel and Kiel University.

Abstract

Clonal reproductive systems abound among marine species, including important ecosystem engineers such as corals and seagrasses. According to conventional wisdom, under a clonal mode of reproduction, (adaptive) evolutionary change is impossible owing to identical replication of modules (shoots /colonies) via mitosis during clonal expansion. Using full-genome data from the model seagrass *Zostera marina* (eelgrass), I show that clones evolve, accumulating genetic differences (SNPs) among modules over time. Using clone mates of known divergence date, a calibration of the somatic genetic clock was possible, allowing, for the first time, absolute dating of clone ages. At the same time, findings of within-clone evolution may explain a long-standing ecological riddle, namely why apparently large and old clones are productive and resilient through time although they feature zero genetic diversity, an observation inconsistent with the notion of genetic diversity determining population viability. An ongoing and difficult next step is the detection of asexual selective sweeps among clonal lineages, for which methods from cancer evolutionary genetics are adapted to free-living clones. Findings of within-clone genetic diversity have implications for defining individuality in modular species, and may require that some conservation genetic rules need modification under clonality.

Population & Species

Microbial assemblages associated to demersal fish species inhabiting the shallow hydrothermal sites of Vulcano Island, Mediterranean Sea

Erika Arcadi[1], Carmen Rizzo[2,3], Pietro Battaglia[4], Maria Giulia Stipa[4,5], Rosario Calogero[4], Pierpaolo Consoli[4], Luca Pagano[4,5], Valentina Sciutteri[4], Franco Andaloro[6], Teresa Romeo[7,8]

[1] Stazione Zoologica Anton Dohrn–Sicily Marine Centre, Department of Biology and Evolution of Marine Organisms (BEOM), Villa Pace, Contrada Porticatello 29, 98167 Messina, Italy.
[2] Stazione Zoologica Anton Dohrn–Sicily Marine Centre, Ecosustainable Marine Biotechnology Department, Villa Pace, Contrada Porticatello 29, 98167 Messina, Italy.
[3] National Research Council, Institute of Polar Sciences (CNR-ISP), Spianata S. Raineri 86, 98122 Messina, Italy.
[4] Stazione Zoologica Anton Dohrn–Sicily Marine Centre, Integrative Marine Ecology Department, Villa Pace, Contrada Porticatello 29, 98167 Messina, Italy.
[5] University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy.
[6] Department of Integrative Marine Ecology, Stazione Zoologica "Anton Dohrn", Sicily Marine Centre, Lungomare Cristoforo Colombo (complesso Roosevelt), 90149 Palermo, Italy.
[7] Stazione Zoologica Anton Dohrn–Sicily Marine Centre, Department of Biology and Evolution of Marine Organisms (BEOM), Via dei Mille 46, 98057 Milazzo, Italy.
[8] National Institute for Environmental Protection and Research (ISPRA) Via dei Mille 46, 98057 Milazzo, Italy.

Aim

Similarly to other marine organisms, fish interacts with microbial communities, establishing either mutualistic or pathogenic relations. The microbial populations associated to fish gut, gills and skin have a strong influence on their host's physiological functions, *i.e.* nutrition, immunity, and growth. The structure of the fish-associated bacterial communities could be affected by host genetics, developmental stage, feeding habits, and environmental conditions. The contribution of these factors on the establishment of fish-bacteria interaction is still unclear. The study aimed at exploring the microbiota from specimens of *Xyrichtis novacula, Serranus scriba, Parablennius sanguinolentus, Trachinus draco,* and *Gobis cobitis*. These fish species commonly inhabit the shallow hydrothermal site of Levante Bay (Vulcano island), where they play an important ecological role as consumers of benthic species.

Methods

To the best of our knowledge, for the first time gut and gill samples were collected from three adult individuals for each species and the associated microbial diversity was assessed by using Illumina MiSeq sequencing of bacterial 16S rDNA region V3-V4.

Results

A total of 58 and 49 bacterial phyla were detected in fish gut and gills, respectively. Protebacteria, Firmicudes, Bacteriodota and Actinobacteria were the most representative groups (> 10% of the total microbial communities). Archaeal communities were less represented (< 1%).

Main conclusion

Overall, while the gill microbial communities were quite similar across the different species, the gut microbial communities showed species-specific variability.

Population & Species

Intra- and transgenerational plasticity and acclimation capability in sea urchins (*Paracentrotus lividus*) under long-term exposure to seawater acidification

Davide Asnicar[1], Marco Munari[2], Maria Gabriella Marin[2]

[1] Aquatic Bioscience, Huntsman Marine Science Centre, St Andrews, NB, Canada.
[2] Department of Biology, University of Padova, Padova, Italy.

Aim

Ocean acidification (OA) responses in marine organisms are often not univocal due to non-uniform exposure setups or different ecological histories of the specimens. To this end, we assessed OA response in sea urchins *Paracentrotus lividus* from sites with different environmental features: the Lagoon of Venice and a pristine coastal area in Adriatic Sea.

Methods

Animals were maintained in laboratory at natural conditions (pH 8.04) and end-of-the-century predicted pH (-0.4 units).

For six months behavioural and physiological (respiration rate, ammonia excretion) endpoints were investigated monthly. After eight months of exposure, the antioxidant and detoxification capabilities were assessed at biochemical level in gonads and digestive tract, whereas immunosurveillance was investigated in the coelomic fluid.

In echinoderms, the larval phase is the most susceptible to environmental stressors. Hence, after 6 months of exposure, embryos were obtained from urchin adults and reared at pH 8.1 and 7.7, assessing larval development after 48 hours.

Results

In coastal urchins, reduced pH had a greater effect compared to lagoonal animals, influencing more heavily both behaviour and physiology. As the exposure continued, animals from both sites were able to acclimate. However, slight effects of pH on the antioxidant and immunological responses were highlighted, with differences between origin sites. The coastal site had higher levels of antioxidant and immune-related biomarkers.

Regarding transgenerational endpoints, larvae reared at low pH showed developmental delay, higher abnormality rate and were smaller than those at pH 8.1, regardless of the parental pH. However, when parents were kept at low pH, the detrimental effects of pH 7.7 were reduced.

Main conclusion

Results suggest adult acclimation and transgenerational beneficial effects in *P. lividus* after long-term exposure to reduced pH, with higher adaptation plasticity in animals from a more variable environment, such as the lagoon, possibly allowing this population to better cope with future pH conditions.

Population & Species

Feeding habits of *Octopus vulgaris* caught off the western Mediterranean, Northern Morocco, with a review of its diet

Salma Bahij[1,2], Hounaida Farah El Idrissi[2], Abdelilah Fahde[1]

[1] Biology and Health Laboratory, Biology Department, Faculty of Sciences Ain Chock, Casablanca, Morocco.
[2] Biology and Ecology Laboratory, Fisheries Department, Regional Center of INRH in Casablanca, Morocco.

Aim

The Moroccan Mediterranean region represents a complex and delicate ecosystem that faces multiple challenges. Among these challenges are intensified fishing activities and changes in climatic and hydrological systems, particularly in the area around the Strait of Gibraltar. These factors pose significant threats to the local populations and the biodiversity of the region. One notable cephalopod species found in this area is the common octopus (Octopus vulgaris), which holds considerable commercial value in global markets, making it an economically important resource for fishing and a crucial player in Morocco's social economy.

Methods

The primary objective of our current research is to investigate the feeding and trophic behavior of Octopus vulgaris. We conducted our study on cephalopods captured off the north coast of Morocco in the Western Mediterranean Sea between 2017 and 2018. Specifically, we examined the stomach contents of 237 common octopuses obtained from the scientific survey called 'Charif El Idrissi.' Out of these, 220 specimens had identifiable food remnants.

To analyze their feeding habits, we employed qualitative and quantitative methods, including numerical frequency indices, frequency of occurrence, weight frequency, vacuity index, and index of relative importance.

Results

Our findings revealed that the common octopuses exhibited opportunistic feeding behavior, as they consumed a diverse range of prey items from three main taxa: Mollusca, Crustacea, and Teleostei. The most significant prey species were Liocarcinus, contributing to 61% of the index of relative importance, along with other decapod crustaceans. Among the various prey groups found in the octopuses' stomachs, mollusks (including bivalves and gastropods) were the most frequently consumed, based on the index of occurrence and importance in both weight and number, followed by crustaceans (specifically brachyurans) and teleosts.

Main conclusion

Furthermore, we observed that the diversity of stomach contents was relatively higher in female octopuses compared to males, as well as in mature specimens compared to immature ones of the Octopus vulgaris species. These findings provide valuable insights into the feeding patterns and ecological dynamics of this cephalopod species in the Moroccan Mediterranean region.

Population & Species

The cradle of diversity: Central Atlantic Ocean hides a mosaic of skate species (Rajiformes)

Valentina Crobe[1], Alice Ferrari[1], Fausto Tinti[1], Alessia Cariani[1]

[1] GenoDREAM, Department of Biological, Geological & Environmental Sciences (BiGeA), University of Bologna, Ravenna, Italy.

Aim

Skates (Rajiformes) represent one of the most intriguing cases of high species richness coupled with extremely conservative morphological and ecological traits. Small differences are described within and between species and increasing cases of cryptic speciation have been recorded among sister and non-sister taxa. More interestingly, this condition has been frequently observed in Central Atlantic Ocean (CAO), along the coasts of Western Africa, between Angola and Senegal, where oceanographic heterogeneities such as current systems, may play a key role for ecological speciation in skates.

Methods

To test the hypothesis that restricted gene flow and genetic divergence within *Raja* species reflect known climate and bio-oceanographic discontinuities the use of multiple mtDNA markers and its integration with nuDNA microsatellite loci has been successfully applied. We estimated the genetic variation and reconstructed phylogenetic relationships, phylogeographic patterns and genetic connectivity in skate species inhabiting the CAO.

Results

The widely distributed *Raja miraletus* species complex counts five deeply distinct lineages three of which co-occur in CAO: the Angolan *Raja* cf. *miraletus*, the Senegalese and sympatric *R. parva* and *Raja* cf. *miraletus*. In addition, an ancient, hidden lineage nesting in the clade *Raja straeleni/R. clavata/R. maderensis* was identified in Angola.

Main conclusions

The complexity of oceanographic system acting in the CAO (i.e., the Benguela Current region and the intertropical Canary current inflowing from the northeast) may have influenced the diversification of the Angolan and Senegalese taxa, boosting the biodiversity of the area, in an unprecedented anthology of species-specific evolutionary histories.

Population & Species

Collective predation of large prey by small-sized cnidarian polyps: individual behavior, population effects, underlying processes

Chiara Gregorin[1,2], Tomás Vega Fernández[2], Luigi Musco[2,3], Stefania Puce[1]

[1] Life and Environmental Sciences Department, Marche Polytechnic University, via Brecce Bianche, 60131 Ancona, Italy.
[2] Department of Integrative Marine Ecology, Ischia Marine Centre, Stazione Zoologica Anton Dohrn, Ischia, Naples, Italy.
[3] Biological and Environmental Sciences and Technologies Department, University of Salento, via Provinciale Lecce-Monteroni, 73100 Lecce, Italy.

Aim

Albeit sessile, cnidarian polyps actively capture large motile prey. Such predatory behavior is not limited to large polyps since small-sized polyps may access large prey by collective protocooperative predation. We investigated the benefits, the behavior, the cues inducing protocooperation, and the pattern of formation of groups by protocooperating polyps.

Methods

First, we provided three diets (large and small, preys and a mixture of both) to *Aurelia coerulea* polyps for 36 days. Periodically, asexual reproduction, growth and interindividual distances were measured to evaluate polyps' fitness and aggregation level. Secondarily, we described the behavior for small and large prey predation, and then provided prey homogenates to serve as cues for protocooperation. Direct observations were carried out for 10 minutes. The number of tentacles contractions were counted as a proxy for feeding excitement.

Results

We reported higher reproduction (+37.5%) and growth (+0.15 mm) in polyps fed with both preys. The optimal group size for protocooperation was 3.05 ± 0.11 individuals. Protocooperative polyps appeared closer compared to polyps eating small prey (–1.71 ± 2.42 mm). The behavior "Active feeding" was significantly related to large prey predation, and it was mainly performed (occurrence >90%) when starved polyps previously fed with large prey were later stimulated with the large prey homogenate. This response decreased after 6 stimulation sessions, indicating habituation.

Main Conclusion

Protocooperation is beneficial for the population and encourages polyps to create groups that may turn into stable aggregations. Predatory behavior could be learnt and mimicked, possibly inducing coordination. Cnidarians are extensively studied for the evolution of physiological and cognitive processes, as sister group of Bilateria. We suggest that cnidarians are the most primitive animals performing group-foraging, this relationship is supposed to involve learning and coordination. These findings are crucial for understanding sociality in the animal kingdom starting from brainless animals.

Population & Species

Simple, yet so complicated: integrative taxonomy reveals *Polyophthalmus pictus* (Annelida: Opheliidae) as a species complex in European waters

Joachim Langeneck[1], **Debora Loprete[2], Alejandro Martínez[3], Luigi Musco[1,4,5], Matteo Putignano[1,4], Andrea Toso[1,4], Sara Verni[2,5], Maria Cristina Gambi[6], Alberto Castelli[2]**

[1] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), U.L.R. di Lecce, c/o DiSTeBA, campus Ecotekne, strada provinciale Lecce-Monteroni, 73100 Lecce, Italy.
[2] Dipartimento di Biologia, Università di Pisa, via Derna 1, 56126 Pisa, Italy.
[3] Consiglio Nazionale delle Ricerche, Istituto di Ricerca sulle Acque, Largo Tonolli 50, 28922 Verbania Pallanza, Italy.
[4] Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (DiSTeBA), Università del Salento, campus Ecotekne, strada provinciale Lecce-Monteroni, 73100 Lecce, Italy.
[5] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.
[6] Istituto Nazionale di Oceanografia e Geofisica Sperimentale (OGS), via Auguste Piccard 54, 34151 Trieste. Italy.

Aim

The occurrence of cryptic and pseudocryptic species is widespread in marine annelids; overlooked diversity can be expected in particular in iconic species that are considered very easy to identify. One of these species is *Polyophthalmus pictus* (Dujardin, 1839), an allegedly cosmopolitan species widespread in shallow, vegetated environments in European waters.

Methods

Polyophthalmus specimens were sampled in several localities in the Mediterranean Sea, mainland European Atlantic coast, and Atlantic Islands, from both photophilous algae (0-15 m) and coralligenous assemblages (50-120 m). The morphological characterisation took into consideration characters traditionally considered informative for the taxonomy of this genus, while the molecular characterisation employed one mitochondrial marker (cytochrome c oxidase subunit I - COI) and one nuclear marker (ITS).

Results

Shallow-water *Polyophthalmus pictus* belonged to four mitochondrial lineages, usually not occurring in syntopy. One lineage, retrieved only in Lebanon, was close to Indo-Pacific *Polyophthalmus* lineages, suggesting a non-indigenous origin in the Mediterranean. Canary and Azores Islands host a distinct mitochondrial lineage from the European mainland, but this distinction cannot be retrieved on the nuclear marker, suggesting a relatively recent diversification of the two lineages. The amplification of COI was not obtained for the majority of the coralligenous *Polyophthalmus*, but based on nuclear data, they belong to different, sometimes very distant lineages. Morphological data traditionally used do not show relevant variation among lineages, with the exception of the number and pattern of lateral ocelli.

Main conclusion

As demonstrated for several marine annelids, *Polyophthalmus pictus* is a species complex. The diversity patterns detected seem to be associated mainly to ecological factors, while biogeography plays a less pronounced role, mostly due to the presence of pelagic dispersal stages in the life cycle of the species.

Population & Species

High within- and between-individual structural variability is related to transposable element insertions and deletions in oysters

Jacopo Martelossi[1], Valentina Peona[2,3], Andrea Luchetti[1], Alexander Suh[4,5], Fabrizio Ghiselli[1]

[1] Department of Biological, Geological and Environmental Science, University of Bologna, Bologna.
[2] Swiss Ornithology Institute; Switzerland.
[3] Swedish Museum of Natural History, Stockholm.
[4] Department of Organismal Biology – Systematic Biology, Evolutionary Biology Centre; Uppsala University, Uppsala.
[5] School of Biological Sciences, University of East Anglia, Norwich, United Kingdom.

Aim

Bivalves are aquatic filter-feeding molluscs characterized by a highly rich and diverse set of transposable elements (TEs) as well as by high levels of heterozygosity. In this study, we used five high-quality, long-read chromosome-level oyster assemblies and whole-genome resequencing data to investigate the impact of TEs in the emergence of within- and between-individual structural variants (SVs). We hypothesize that transposons can be an overlooked but important source of genetic variability both within and between individuals in bivalves and that they can contribute to population differentiation.

Methods

We combined multiple long-read pipelines and simulations to characterize high-confidence within-individual SVs. We have then estimated the role of TEs in their emergence. To characterize between-individual SVs we used 120 publicly available *Crassostrea ariakensis* short read resequenced samples encompassing the entire Chinese coasts. This SV set was used in classical population genomics analyses to detect population structure, differentiation, and putative variants under selection.

Results

Between 4% and 14% of oyster genomes exhibit structural variability between homologous chromosomes. Most of these regions are enriched in transposable elements, including both putative TE insertions and deletions, but are depleted of host genes. Similar results were obtained when analyzing SVs between individuals. Population structure based on more than 60,000 high-confidence SVs reflects the geographic origin of samples, reflecting what can be recovered using SNPs. Among these SVs, we identified multiple loci under putative diversifying selection between southern and northern populations.

Main conclusions

Applying state-of-the-art methods and simulations we suggest an improved workflow for within-individual SVs calling. We discovered that transposon can be an important hotspot of genetic variability in bivalves providing raw material for potential local adaptations. Our analyses highlight the importance of considering structural variants in population genomics analyses in bivalves, especially with the rise of the pan-genomic era.

Population & Species

Shark microbiome analysis demonstrates unique microbial communities in two distinct Mediterranean Sea shark species

Francesco Montemagno[1], **Chiara Romano**[2], **Angelina Cordone**[1], **Olga De Castro**[1,8], **Sergio Stefanni**[3], **Emilio Sperone**[2] **and Donato Giovannelli**[1,4,5,6,7]

[1] Department of Biology, University of Naples Federico II, Naples, Italy.
[2] Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende (CS), Italy.
[3] Stazione Zoologica Anton Dohrn, Naples, Italy.
[4] National Research Council – Institute of Marine Biological Resources and Biotechnologies - CNR-IRBIM, Ancona, Italy.
[5] Department of Marine and Coastal Science, Rutgers University, New Brunswick, NJ, USA.
[6] Marine Chemistry & Geochemistry Department - Woods Hole Oceanographic Institution, MA, USA.
[7] Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan.
[8] Botanical Garden, University of Naples Federico II, Naples, Italy.

Aim

Our knowledge of the role of fish microbiome in their health has been steadily increasing in the last decade, especially for species of commercial interest. Conversely, relatively few studies focus on the microbiome of wild fishes, especially apex predators like sharks, due to lower economic interest and to greater difficulty in obtaining samples. Studies investigating microbiome differences between diverse anatomical locations of sharks are limited, and the majority of available studies are focused on the microbial diversity present on the shark teeth, with the aim of preventing infections due to the bite of these animals or evaluating the presence of certain pathogens in healthy or diseased specimens. Here, we investigated the skin, mouth, gills, and cloaca microbiome of five individuals of two phylogenetically distant shark species (*Prionace glauca* and *Somniosus rostratus*), to get a better understanding of the diversity of the microbiome of these animals, how it changes throughout different anatomical locations and how much it is influenced and determined by the ecology and evolutionary relationship host-microbiome.

Methods

To confirm the taxonomy of the sharks under study, we barcoded the specimens by sequencing the mtDNA COI from a biopsy of their skin. We also used 16S amplicon sequencing to investigate microbiome differences across the different anatomical locations and between the two shark species.

Results

Microbial diversity analysis reveals that partially overlapping microbiomes inhabit different body parts of each shark species, while the communities are distinct between the two species.

Main conclusion

Our results suggest that sharks' microbiome species-specific differences are controlled by the ecology of the shark species. This is the first study comparatively analyzing the microbiome diversity of different anatomical locations in two shark species of the Mediterranean Sea.

Population & Species

A fiery nudibranch: the amazing connection between *Spurilla neapolitana* (Delle Chiaje, 1841) (Mollusca, Heterobranchia) and its symbiotic unicellular algae

Bruna Oršanić[1], Andrea Toso[2], Michele Solca[3], Paolo Mariottini[4], Chiara Papetti[1], Giulia Furfaro[2,5]

[1] Department of Biology, University of Padova, Padova, Italy. bruna.orsanic@studenti.unipd.it; chiara.papetti@unipd.it.
[2] Department of Biological and Environmental Sciences and Technologies – DiSTeBA, University of Salento, I-73100 Lecce, Italy. giulia.furfaro@unisalento.it.
[3] Museo di Biologia Marina "Pietro Parenzan", Via Vespucci 13/17, 73010 Porto Cesareo, Lecce, Italy. michelesolca@gmail.com.
[4] Department of Science, University of "Roma Tre", Viale G. Marconi 446, 00146 Rome, Italy. paolo.mariottini@uniroma3.it.
[5] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy.

Aim

Nudibranchs (Gastropoda) are known for their extraordinary ability to obtain structures, cells or chemical compounds from their preys and include them in their own tissues or cells. These unique strategies co-evolved with the loss of the shell in the adult stage. In this framework, symbiotic relationships were already reported between some nudibranchs and unicellular algae but currently the pattern of distribution inside the body of the host is still lacking. To fulfil this gap of knowledge, *Spurilla neapolitana* (Delle Chiaje, 1841), a Mediterranean facelinid preying mainly on *Anemonia* Risso, 1827 anemones known to host symbiotic algae, was investigated using fluorescence to confirm the presence of the symbiont and describe its distribution. Furthermore, molecular techniques were used to confirm this symbiotic association and investigate on the systematic of the zooxanthellae involved.

Methods

Seven *S. neapolitana* and two *A. sulcata* were collected from Taranto (Ionian Sea), observed and photographed alive using the fluorescence microscope before have being store in EtOH 96% for further molecular analyses. DNA was extracted from both nudibranchs and anemone and four molecular markers (COI, 16S, H3, ITS2) used for barcoding analysis.

Results

Fluorescence clearly confirmed the presence of the symbiont in *S. neapolitana* and revealed a widespread distribution throughout almost all its dorsal surface. Furthermore, while the common markers confirmed the species identity, the nuclear ITS2 showed a double band within the nudibranch sample. This result is promising since confirm the presence, in the same tissues, of both host and symbiont thanks to the different length of ITS2 from different organisms.

Main conclusion

This is the first study describing the distribution pattern of symbiotic algae inside the body of its nudibranch host. Furthermore, firstly in a nudibranch, this symbiotic relationship was investigated using the ITS2 marker to reveal simultaneously the presence of the host and its symbiont.

Population & Species

Pangenomic architectures are more widespread than originally thought in marine invertebrates: first evidence of gene presence/absence variation in the periwinkle *Littorina fabalis*

Marco Sollitto[1,2], Rui M Macieira De Faria[3], Marco Gerdol[1]

[1] Department of Life Sciences, University of Trieste, Italy.
[2] Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Slovenia.
[3] CIBIO, University of Porto, Portugal.

Aim

Recent studies have evidenced that several bivalve species display an unusual pangenomic architecture, characterized by widespread hemizygosity and remarkable presence/absence variation (PAV) phenomena, which can affect several thousand protein-coding genes. Although these features might have a critical impact on phenotype, with important implications for local adaptation, this theme has been only marginally investigated in gastropods. Here, we preliminarily investigated the occurrence of gene PAV in the periwinkle *Littorina*, a well-established model for evolutionary biology studies in marine environments.

Methods

Following the refinement and annotation of the draft genome of *Littorina fabalis*, we analyzed the whole-genome resequencing data of 12 individuals belonging to two distinct ecotypes (“large” and “dwarf”), looking for signatures of PAV that might help to define the “openness” of the periwinkle pangenome and investigate the involvement of dispensable genes in specific biological pathways. *In silico* predictions were further validated through PCR.

Results

Gene PAV affected a non-negligible fraction of protein-coding genes (about 2000, ~6% of the total) in *L. fabalis*. Although structural variation was less prevalent than in bivalves, these findings indicate the presence of a pangenomic architecture that likely includes several hundred dispensable genes not represented in the reference genome. Interestingly, the accessory fraction of the periwinkle pangenome was significantly enriched in genes belonging to the AIG1 family of small GTPases, which have been previously implicated in stress adaptation and resistance towards infection in other mollusks. Moreover, PAV patterns correlated with observed phenotype, suggesting that dispensable genes may contribute to local adaptation.

Main conclusion

These findings confirm the occurrence of gene PAV in non-bivalve mollusks and highlight the need to adopt a paradigm shift towards the use of a pangenome instead of a monoploid assembly as a reference for genomic studies in this species.

Population & Species

Unveiling cryptic diversity in a pelagic sea slug (Gastropoda, Heterobranchia)

Andrea Toso[1], Marcella D'Elia[2], Michele Solca[3], Egidio Trainito[4], Paolo Mariottini[5], Giulia Furfaro[1,6]

[1] Department of Biological and Environmental Sciences and Technologies - DiSTeBA, University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. andrea.toso@unisalento.it; giulia.furfaro@unisalento.it.
[2] Department of Mathematics and Physics "Ennio De Giorgi", University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. marcella.delia@unisalento.it.
[3] Museo di Biologia Marina "Pietro Parenzan", Via Vespucci 13/17, 73010 Porto Cesareo, Lecce, Italy. michelesolca@gmail.com.
[4] Genoa Marine Centre-Stazione Zoologica Anton Dohrn, Istituto Nazionale di Biologia, Ecologia e Biotecnologie Marine, Villa del Principe, Piazza del Principe 4, 16126 Genoa, Italy. et@egidiotrainito.it.
[5] Department of Science, University of "Roma Tre", Viale G. Marconi 446, 00146 Rome, Italy. paolo.mariottini@uniroma3.it.
[6] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy. giulia.furfaro@unisalento.it.

Aim

Nudibranchs are a group of specialized molluscs characterized by an adult stage without the shell and the parallel evolution of unique alternative defensive strategies. They are characterized by a great adaptative radiation having colonised almost all the marine habitats from deep waters to sea surface, where only few specialized Cladobranchia can live. This is the case of the worldwide rafting *Fiona pinnata* (Eschscholtz, 1831) mainly feeding on the goose barnacles anchored on floating substrates. Even if this nudibranch has been investigated by Trickey et al. (2016), who unveiled a possible species complex, this case was not resolved and their results forgotten, so that *F. pinnata* species complex is currently accepted as a valid species. Considering that Mediterranean specimens have never been studied, we investigated this unsettled case through an integrative approach, adding samples from all the Italian seas (*i.e.* Tyrrhenian, Ionian and Adriatic Seas).

Methods

Bayesian and Maximum likelihood analyses were performed on four molecular markers (COI, 16S, ITS2, H3) on a broader dataset including unpublished Mediterranean and GenBank sequences from all over the world. Additionally, anatomical analysis of the reproductive system, optical and SEM observations of the buccal apparatus, and morphological larvae development, were performed.

Results

Morphological and molecular results confirmed all the Mediterranean individuals belong to a species well separated from the 'true' *F. pinnata* and consequently, we propose reinstating *Fiona marina* (Forsskål, 1775) as a valid species.

Main conclusion

The case of *F. pinnata* species complex was resolved, and the congeneric *F. marina* reinstated as valid. These results are particularly intriguing and highlight the integrative taxonomy approach as powerful to unravel cryptic diversity. Moreover, this study confirmed the need to analyse Mediterranean specimens especially when these were used in the original descriptions of the species.

Population & Species

Genomic reconstruction of the divergence dynamics of two scallop species

David L. J. Vendrami[1], Joseph I. Hoffman[1,2], Craig S. Wilding[3]

[1] Department of Animal behaviour, Bielefeld University, Bielefeld, Germany. David L. J. Vendrami: david.vendrami@uni-bielefeld.de
[2] British Antarctic Survey, Cambridge, United Kingdom. Joseph I. Hoffman: joseph.hoffman@uni-bielefeld.de
[3] School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, United Kingdom. Craig S. Wilding: C.S.Wilding@ljmu.ac.uk

Aim

The great scallop (*Pecten maximus*) and the Mediterranean scallop (*P. jacobaeus*) are two European scallop species whose distribution abut at the Almeria-Oran front. These species were initially believed to have diverged shortly after the Messinian salinity crisis, around 5 million years ago (mya), but a number of genetic-based studies challenged this view by providing measures of genetic divergence indicative of a more recent split. Here, we use a genomic approach to reconstruct the divergence history of these two species and uncover the potential determinants that led to their differentiation.

Methods

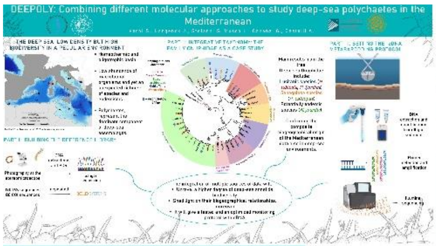
Our analyses were based on RAD sequencing data obtained from both scallop species and a chromosome-level reference genome. Firstly, we implemented demographic modelling to compare different speciation scenarios. Secondly, we calculated absolute and relative measures of genetic divergence between the two species to characterize levels of differentiation along the *Pecten* genome. Finally, we characterized picks of divergence to uncover the genomic context where they were located.

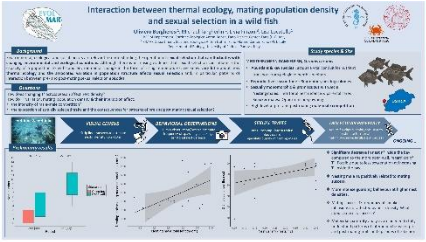
Results

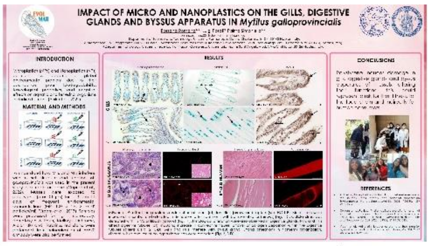
The two scallop lineages appeared to have diverged in allopatry around one mya. This was followed by secondary contact, which occurred at the end of the last glacial period. The *Pecten* genome was non-uniformly affected by gene flow during secondary contact and picks of divergence were found in genomic regions showing low recombination rates. Moreover, contrasting patterns of LD decay were detected for the two species, hinting at the presence of chromosomal inversions. Highly differentiated genomic regions were enriched for immune-related processes, mRNA modification and molybdenum metabolism and thus appeared to be adaptively important.

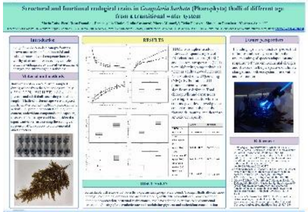
Main conclusion

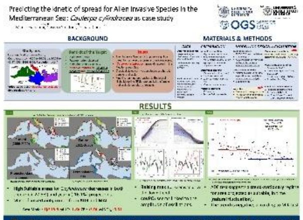
The great and the Mediterranean scallops diverged more recently than initially thought. Divergence accumulated over an initial period of allopatry and peaks of adaptively important differentiation were maintained despite secondary contact in genomic regions that are reproductively isolated.


DEEPOLY: Combining different molecular approaches to study deep-sea polychaetes in the Mediterranean

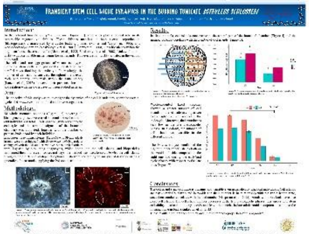

Interaction between thermal ecology, mating population density and sexual selection in a wild fish

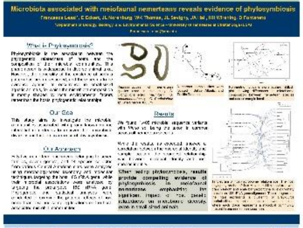

IMPACT OF MICRO AND NANOPLASTICS ON THE GILLS, DIGESTIVE GLANDS AND BYSSUS APPARATUS IN Mytilus galloprovincialis

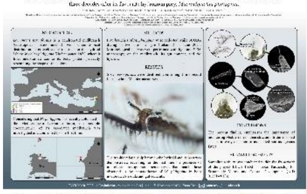


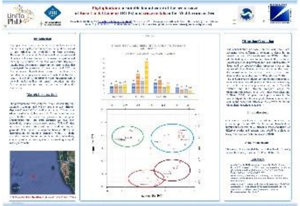


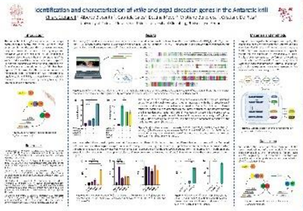
Posters

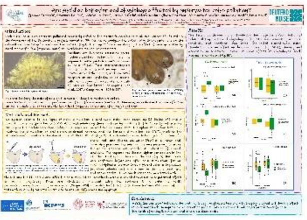


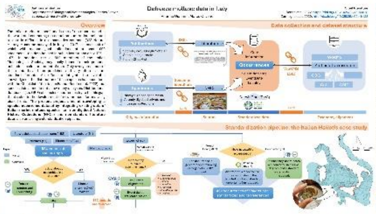

Microbiota associated with meiofaunal nemerteans reveals evidence of phylosymbiosis

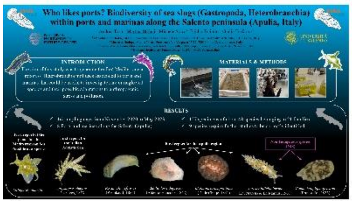


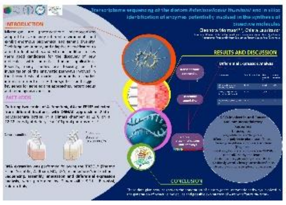


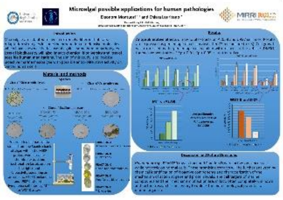


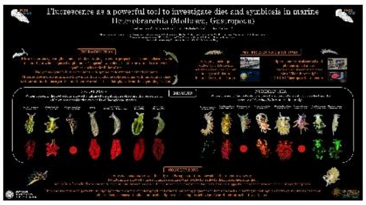





Who likes ports? Biodiversity of sea slugs (Gastropoda, Heterobranchia) within ports and marinas along the Salento peninsula (Apulia, Italy)






Fluorescence as a powerful tool to investigate diet and symbiosis in marine Heterobranchia (Mollusca, Gastropoda)

Adaptation

Interaction between thermal ecology, mating population density and sexual selection in a wild fish.

Oliviero Borgheresi[1], Michael Tangherlini[2], Livia Pinzoni[3], Lisa Locatello[1]

[1] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Fano Marine Center, Fano (PU), Italy.
[2] Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Fano Marine Center, Fano (PU), Italy.
[3] Department of Biology, University of Padova, Padova, Italy.

Aim

Sexual selection is a powerful evolutionary agent responsible for the extraordinary variability in reproductive phenotypes ranging from fascinating and unusual displays to cryptic traits whose function is only uncovered by careful study. Pre- and post-mating episodes of sexual selection combine and interact to determine overall male reproductive fitness, and emerging evidence has been emphasising the role of sexual selection in influencing the capacity of a population to withstand environmental change, such as temperature variation. However, we still know very little about how thermal ecology influences sexual selection and, in particular, patterns of interaction between pre- and post-mating sexual selection episodes. Our study aims to fill this gap, focusing on one of the most abundant fish species in the rocky littoral environment of the Mediterranean Sea, the damselfish *Chromis chromis*.

Methods

We explored the influence of temperature variation across the reproductive season on: nest density and competition for mating (through scuba visual census and behavioural observations); expression of male traits that are important in pre- and post-mating episodes of sexual selection (through morphological measures and physiological analyses on the ejaculate); the resulting pre- and post-mating reproductive success (through molecular paternity analyses).

Results

Here we show our preliminary results on the relation between temperature variation across season and nest density at different depths, and how this influences the intensity of male-male competition and the expression and relative importance of pre- and post-copulatory sexual traits in determine overall male reproductive fitness.

Main conclusion

Our study contributes to extend the comprehension of evolutionary interaction between thermal ecology and sexual selection under changing environmental conditions and to better predict potential adaptation to future scenarios.

Adaptation

Transcriptome sequencing of the diatom *Asterionellopsis thurstonii* and in silico identification of enzymes potentially involved in the synthesis of bioactive molecules

Eleonora Montuori[1,2], Chiara Lauritano[2]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy; eleonora.montuori@studenti.unime.t.
[2] Ecosustainable Marine Biotechnology Department, Stazione Zoologica Anton Dohrn, Via Acton 55, 80133 Naples, Italy; chiara.lauritano@szn.it.

Aim

Microalgae are photosynthetic microorganisms adapted to live in very different environments and exhibit enormous biochemical and genetic diversity. Their long evolutionary and adaptive diversification to a multitude of habitats and extreme conditions makes them good candidates for the discovery of new molecules with potential human applications. Recently, many studies are focusing on the investigation of the enzymatic pathways involved in the biosynthesis of marine compounds of market interest in order to clarify their synthesis and suggest key genes for gene editing approaches, heterologous and homologous expression.

Methods

We used an RNA-sequencing approach to de novo sequence the full transcriptome of two strains, A4 and FE355, of the diatom *A. thurstonii*, sampled from two different locations, and cultured in both control and phosphate starvation conditions. Differential expression analysis was also performed.

Results

We in silico identified transcripts coding L-asparaginase I, polyketide cyclase/dehydrase, bifunctional polyketide phosphatase/kinase, 1-deoxy-D-xylulose-5-phosphate synthase (fragment), inositol polyphosphate 5-phosphatase INPP5B/F, catechol O-Methyltransferase, digalactosyldiacylglyc-erol synthase (DGD1), 1,2-diacylglycerol-3-beta-galactosyltransferase and glycerolphosphodiester phosphodiesterase. These are enzymes potentially involved in the synthesis/degradation of compounds with anti-cancer and immunomodulatory properties. Some of them were differentially expressed as well.

Main conclusion

These data give new insights on the annotation of diatom genes, enzymatic pathways involved in the generation of bioactive molecules and possible exploitation of *Asterionellopsis thurstonii*.

Adaptation

Identification and characterization of *vrille* and *pdp1* circadian genes in the Antarctic krill

Chiara Stefanelli[1], **Alberto Biscontin**[2], **Gabriele Sales**[1], **Bettina Meyer**[3], **Cristiano Bertolucci**[4], **Cristiano De Pittà**[1]

[1] Department of Biology, University of Padova, Padova, Italy.
[2] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy.
[3] Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Oldenburg, Germany.
[4] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy.

Aim

The Antarctic krill *Euphausia superba* is a pelagic organism with a central role in the Southern Ocean ecosystem. During the ages, this crustacean developed daily and seasonal rhythms in its physiology and behaviour which are regulated by an endogenous molecular clock. The main components of the krill's circadian clock have been characterized, revealing an ancestral clock sharing both mammalian and insect features. Recently, the rhythmic expression of krill's orthologue of *D. melanogaster* transcription factor VRI has been reported, suggesting the possible conservation of a previously characterized modulation loop consisting of two transcriptional regulators, VRI and PDP1. In *Drosophila*, these factors are known to modulate CLOCK expression by binding to a V/P-box located in its gene promoter.

Methods

Using *D. melanogaster* homologues as inputs, the coding sequences of three different isoforms of *pdp1* were identified in the krill's transcriptome database KrillDB[2]. These sequences were cloned into specific vectors for the expression in *Drosophila* cells. Finally, a luciferase assay was performed to investigate the interaction of VRI and PDP1 with the V/P-box of *Drosophila clock* gene promoter. In addition, *vrille* and *pdp1* isoforms expression levels were assessed in five different krill tissues through qRT-PCR.

Results

The luciferase assay revealed the opposite action of these two factors, with VRI as inhibitor and PDP1 as activator of *clock* transcription. This result was achieved with all the tested isoforms with different degrees of action. At the same time, we characterized the expression patterns of *vrille* and *pdp1* in different krill's tissues in order to test the different isoforms tissue specificity.

Main conclusion

We discovered and characterized a new component of the Antarctic krill clock, a feedback loop composed by the transcriptional factors PDP1 and VRI, regulating CLOCK expression. This study will pave the way to complete the functional characterization of krill's clock machinery.

Biodiversity

Transient stem cell niche dynamics of the budding tunicate *Botryllus schlosseri*

Federico La Torre[1], Virginia Vanni[2], Davide Asnicar[3], Federico Caicci[1], Giacomo Sabbadin[1], Emanuela De Lisa[1], Lucia Manni[1]

[1] Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35131 Padova.
[2] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK.
[3] Aquatic Bioscience, Huntsman Marine Science Centre, 1 Lower Campus Road, St Andrews, New Brunswick, Canada E5B 2L7.

Aim

Tunicates, the sister group of vertebrates, exhibit stem cell-based processes as adaptation to environmental changes. Stem cells are able to self-renew and differentiate into different cell types, allowing growth and repair of damaged tissues. In the colonial tunicate *Botryllus schlosseri*, stem cells are involved in asexual reproduction and regeneration, and home in adult zooid niches. A colony, composed of adult individuals and their buds, cyclically undergoes a generational change: adult zooids are resorbed and substituted by their buds. During this phase, stem cells leave their niches to colonize those of the new adults. Here, we describe the dynamics of the cell islands, a *B. schlosseri* niche, throughout the asexual cycle. Cell islands are groups of phagocytes and stem cells, located in the ventral body wall.

Methods

Colonies fixed at different phases were used to count cell islands in each adult, and analyse their location. Moreover, 39 adults from three colonies were studied *in vivo* throughout the asexual cycle. *Escherichia coli* fluorescent bioparticles were microinjected into their vasculature. Circulating phagocytes turned fluorescent after bioparticle ingestion and homed in cell islands, making them detectable. Cell islands were counted in each individual every 24 hours and their location were annotated. Data were statistically analysed to infer the dynamicity of cell islands during the asexual cycle and in different physiological conditions.

Results

Cell islands exhibited varying locations and differed in number in genetically identical individuals. In single individuals, their number showed a positive correlation with the physiological condition and changed throughout the asexual cycle.

Main Conclusion

Cell islands are a dynamic niche, varying in relation to the asexual cycle. Healthy zooids with more buds show also more cell islands. The variations observed in genetically identical individuals suggest that environmental factors play a role in determining the number and position of cell islands.

Biodiversity

Microalgal possible applications for human pathologies

Eleonora Montuori[1,2], Chiara Lauritano[2]

[1] Department of Chemical, Biological, Pharmacelauritanoutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy. eleonora.montuori@szn.it.
[2] Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Via Acton 55, 80133 Napoli, Italy.

Aim

Marine microalgae are photosynthetic eukaryotic organisms that contribute at 40% of the global productivity. They are characterized by a great biodiversity in terms of species able to live in extremely different habitats thanks to their adaptation versatility. Adaptation strategies include the production of a series of molecules with defensive capacities. These compounds have been shown to exert activities useful against some human pathologies. Loss of biodiversity will also impact chemical biodiversity and loss of possible human medications. Due to the high incidence of cancer, infectious diseases, antibiotic resistance and the insurgence of other human pathologies, the aim of this study is to look for bioactive marine microalgae with possible antiproliferative activity for human health applications.

Methods

Six freeze-dried microalgal pellets were extracted with a methanolic extraction and the organic extracts were fractionated with Solid Phase Extraction chromatography method. Raw extracts and fractions were tested on human melanoma and lymphoma cells.

Results

For each extract, five fractions named fraction A-E, enriched of specific compounds, were obtained. Primary metabolites extracted in each fraction were amminoacids and saccharides (fraction A), nucleosides (fraction B), glycolipids and phospholipids (fraction C), sterols and free fatty acid (fraction D) and triglycerides (fraction E), respectively. Specific extracts and fractions showed antiproliferative activity, without affecting normal cells used as control.

Main conclusion

These promising extracts will be further considered for successive analyses, such as chemical identification of bioactive components and characterization of the mechanism of action at gene and protein levels. The cell targets of marine compounds and their mechanism of action are, in fact, often completely unknown and further research is necessary to unlock the biotechnological potential of marine organisms.

Biodiversity

Fluorescence as a powerful tool to investigate diet and symbiosis in marine Heterobranchia (Mollusca, Gastropoda)

Andrea Toso[1], Federica Casaluce[1], Michele Solca[2], Giulia Furfaro[1,3]

[1] Department of Biological and Environmental Sciences and Technologies - DiSTeBA, University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. andrea.toso@unisalento.it; federica.casaluce1@studenti.unisalento.it; giulia.furfaro@unisalento.it.
[2] Museo di Biologia Marina "Pietro Parenzan", Via Vespucci 13/17, 73010 Porto Cesareo, Lecce, Italy. michelesolca@gmail.com.
[3]National Biodiversity Future Center (NBFC), 90133 Palermo, Italy. giulia.furfaro@unisalento.it.

Aim

Chlorophyll fluorescence is today used as a sensitive, in vivo probe of photosynthetic function and it is commonly used in different fields as well as to investigate the effect of climate change in marine habitats. It is a fast, easy, and non-invasive method but despite its simplicity, it has never been used in Mediterranean Heterobranchia to explore diet and symbiosis, even if these molluscs are known for their symbiotic relationships with unicellular algae or their ability to incorporate into their own cells the chloroplasts from the algae they feed on. For these reasons, this technique was applied to different Heterobranchia species characterized by own ecology and trophic strategies.

Methods

Six Sacoglossa and seven Nudibranchia were collected using scraping and scuba dive techniques from different localities along the Salento peninsula (Apulia). All the specimens were observed and photographed using a Nikon SMZ25 stereomicroscope fitted with NIS-Elements imaging software and the fluorescence investigated using Nikon intensilight C-HGFI fiber optic fluorescence.

Results

A high presence of fluorescence was reported in all the Sacoglossa analysed except *Placida cremoniana* (Trinchese, 1892). Regarding nudibranchs, results showed a more complex scenario that perfectly reflects the heterogeneous diet characterizing each nudibranch family. However, the case of *Favorinus branchialis* (Rathke, 1806) is noteworthy since it was known to feed on nudibranch's eggs but the discover of unicellular algae in its tissues suggests it may feed also on other unreported prey containing zooxanthellae.

Main conclusion

Fluorescence revealed powerful to highlight the presence of chlorophyll and other fluorescing pigments in heterobranchs. Additionally, it allowed to distinguish between symbiosis based on chloroplasts or zooxanthellae. Finally, results on the pattern of distribution of these exogenous organelles or cells in the body of the hosts can fill some gaps of knowledge still existing on these interesting but yet poorly known biological abilities.

Biodiversity

Who likes ports? Biodiversity of sea slugs (Gastropoda, Heterobranchia) within ports and marinas along the Salento peninsula (Apulia, Italy)

Andrea Toso[1], Martina Stifani[1], Michele Solca[2], Egidio Trainito[3], Giulia Furfaro[1,4]

[1] Department of Biological and Environmental Sciences and Technologies - DiSTeBA, University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. andrea.toso@unisalento.it; martina.stifani@studenti.unisalento.it; giulia.furfaro@unisalento.it.
[2] Museo di Biologia Marina "Pietro Parenzan", Via Vespucci 13/17, 73010 Porto Cesareo, Lecce, Italy. michelesolca@gmail.com.
[3] Genoa Marine Centre-Stazione Zoologica Anton Dohrn, Istituto Nazionale di Biologia, Ecologia e Biotecnologie Marine, Villa del Principe, Piazza del Principe 4, 16126 Genoa, Italy. et@egidiotrainito.it.
[4] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy. giulia.furfaro@unisalento.it.

Aim

Mediterranean Sea is a hot spot of Heterobranchia diversity; in fact, more than 363 taxa are reported for this semi-closed basin, 281 of which are present in the Italian waters. Even if an increasing effort have been done to study the Mediterranean fauna, almost all the available checklists focused on coastal natural areas while artificial marine sites as ports and marinas, potentially interesting also for the early detection of non-indigenous species (NIS), remain unexplored yet. In fact, most of the previous studies reported few species inhabiting these environments but the list of associated fauna is still lacking. To fulfil this gap of knowledge, the sea slugs inhabiting ports and marinas along the Salento peninsula were investigated and the species list produced.

Methods

Brushing and scuba diving techniques were used to collect heterobranchs from eight stations along the Salento peninsula. The benthic detritus was observed under the stereomicroscope and sorting of the species carried out. Individuals collected were photographed and stored in EtOH 96%.

Results

A total of 158 specimens was recorded belonging to at least 49 species. Among these, only two are considered NIS while for nine taxa further analysis are needed to correctly assign them at the species level. However, the presence of *Stiliger* cf. *auarita* is noteworthy as new species and genus for the entire basin. Furthermore, 49 representative drawings were produced per each species.

Main conclusion

Studying the Heterobranchia associated to ports and marinas revealed useful to unravel rare and neglected species and could constitute a valid support for studying Heterobranchia diversity and for the early warning of NIS. Furthermore, bridging some knowledge gaps on these highly impacted environments may constitute an opportunity to investigate the adaptive dynamics shown by these molluscs to respond to the anthropic impacts acting on these sites.

Conservation

Phytoplankton: a suitable bioindicator of the occurrence of Rare Earth Elements (REEs) and trace metals in the Mediterranean Sea

Ermelinda Del Buono[1,2] Nicola Nurra[1,3], Alessandra Griglione[4], Marco Battuello[1,3], Rocco Mussat Sartor[1,3], Livio Favaro[1], Guillem Mateu Vicens[2], Giovanna Berti[4], Maria Cesarina Abete[4], Stefania Squadrone[4]

[1] Department of Life Sciences and Systems Biology - University of Turin, Accademia Albertina Street 13,10123 Turin, Italy.
[2] University of the Balearic Islands, Ctra. Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain.
[3] Pelagosphera, Marine Environmental Services Cooperative, Umberto Cosmo Street 17/bis, 10131 Turin, Italy.
[4] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Via Bologna148, 10154 Turin, Italy.

Aims

Phytoplankton plays a key role in marine food webs and in the neritic and pelagic ecosystems as, being at the base of it, can influence the availability of trace elements. However, above a certain level essential and non-essential elements could cause damage to the marine ecosystem, and reaching higher levels of the food web could also cause toxicity risks for humans. The study aims to investigate the presence of REEs and trace metals at different depths in phytoplankton populations from the Ligurian Sea to understand if these components, which represent a truly important link in the food web, can be used as bioindicators to detect the presence of these elements in the marine ecosystem.

Methods

The phytoplankton was collected in the four seasons (Spring, Summer, Autumn, and Winter 2021) at two different depths (-30 m and -50 m). with a mesh size of 20- microns (diameter of 40 cm), frozen at -18 °C, and stored in dark glass bottles. Trace and rare earth elements were analyzed by inductively coupled plasma spectrometry (ICP-MS) after microwave mineralization with nitric acid and oxygen peroxide.

Results

The results show that trace elements and REEs have a higher concentration at 30 m of depth, apart from Fe, Zn, Mn, and V which appear to be more concentrated at 50 m. Moreover, we have also noticed that both trace and REEs were recorded at the highest levels in the winter season and lowest in spring.

Main Conclusion

The presence of inorganic compounds in the first meters of depth can be attributed to some biochemical processes, such as the absorption of phytoplankton in surface waters and the decomposition and demineralization of organic material in deep waters. Fe and Zn have higher concentrations than other elements as they are involved in the fixation of C and N and in the electron transport chain for photosynthesis.

Conservation

Predicting the kinetic of spread for Alien Invasive Species in the Mediterranean Sea: Caulerpa cylindracea as a case study

Marco Fianchini[1,2], **Cosimo Solidoro**[2], **Donata Canu**[2]

[1] Department of Life Sciences, University of Trieste, 34127, Trieste, Italy.
[2] National Institute of Oceanography and Applied Geophysics, OGS, Borgo Grotta Gigante, 42/c, 34010 Sgonico, Trieste, Italy.

Aim

Alien Invasive Species (AIS) pose a significant threat to marine ecosystems, and can cause irreversible damage to the environment, native species, and human welfare. One of these species, *Caulerpa cylindracea*, has rapidly spread in the Mediterranean Sea, affecting biodiversity and ecosystem functioning. In this study, the future spread of *C. cylindracea* in the Mediterranean Sea under changing climatic conditions is predicted.

Methods

Using high-resolution oceanographic and environmental data derived from IPCC-AR5-RCP8.5 based MEDSEA-PHYSICS and MEDSEA-BIOGEOCHEMISTRY simulations and EMODnet Seabed Habitats. Species occurrences data were derived from the Reef Check Med Dataset and EASIN - European Alien Species Information Network dataset.

The dataset contained physical, biogeochemical and biological data at a spatial resolution of 1/24 degree and a yearly time resolution from 2000 to 2050. A Maximum Entropy (MaxEnt) spatial distribution modeling framework was implemented, using the best available techniques, to create predictive models for the future spreading of this invasive alien species. In particular we used six commonly used performance metrics (AICC, AUC, SEDI, CBI, OR.10p, AUCDIFF) to evaluate the model performances of 1240 models configurations obtained by varying models parameters and combine the results.

Results

Our results show the potential distribution of *C. cylindracea* under current and future climatic conditions.

Main conclusion

Results suggests a plateau-like invasion kinetic (sometimes referred as natural fluctuation model) in the currently suitable areas, with no sign of widespread suitability increase in the near future.

Conservation

Defreeze mollusc data in Italy

Arianna Giannini[1], Marco Oliverio[1]

[1] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Italy.

Aim

The strategic location and hydrological-climatic history of the Mediterranean Sea make it a biodiversity hotspot, prompting extensive studies in this region. However, distributional data for Mediterranean taxa are scattered across various sources and formats, including articles, datasets, and non-digitized biological collections, yielding a loss in information. Retrieving and harmonizing these scattered data could facilitate their use for biodiversity research and conservation.

The aim of this work is to provide a standardized pipeline to integrate these dispersed dataset of Italian marine species occurrences, using molluscs as a proxy.

Methods

Data were gathered from two main sources: published literature and biological collections. Occurrence records were defined as those containing at least an identification and a location. The harmonization process involved four key steps: 1) taxonomy updating, 2) terminology and structure standardization, 3) conversion of dates to ISO 8601 format, and 4) transforming textual localities into spatial points with coordinates and a degree of uncertainty, following a standardized workflow. Each record then underwent a quality check to assess its usability.

Results

Our efforts yielded over 30,000 standardized records of marine molluscs in Italian waters. These records encompassed newly digitized specimens from 10 different institutions and private collectors, as well as data extracted from more than 70 published studies. The most represented groups were gastropods and bivalves. The dataset also included information on alien, rare, and endemic species.

Main conclusion

The integration of published and unpublished data may provide a comprehensive spatial and temporal overview of Italian marine malacofaunas. This approach can shed light on under-sampled areas and taxa, guiding potential avenues for future studies.

Conservation

Are Ascidians behavior and physiology affected by underwater noise pollution?

Giacomo Sabbadin[1], Emanuela de Lisa[1], Federico la Torre[1], Loriano Ballarin[1], Chiara Anselmi[2], Giovanni Zambon[3], Alessandro Bisceglie[3], Emanuele Asnaghi[3], Lucia Manni[1]

[1] Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35131 Padova.
[2] Stem Cell Biology Regenerative Medicine Institute, Stanford University 450 Jane Stanford Way Stanford, CA 94305-2004.
[3] Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano-Bicocca Piazza dell'Ateneo Nuovo, 1 - 20126, Milano.

Aim

Ascidians are tunicates possessing mechanoreceptors potentially able to respond to water particle motions caused by underwater noise. These mechanoreceptors include both epidermal neurons, mainly located around the siphons, and receptors on oral tentacles, called coronal cells. CCs are homologues of vertebrate hair cells, therefore potentially sensible to noise. The latter is known to negatively affect some marine vertebrates. However, the ability of most invertebrates to tolerate and adapt to this environmental stressor are still largely unknown. Within the DeuteroNoise project (financed by JPI Oceans), we analyzed the effects of anthropogenic low-frequency noises, *i.e.* those generated by maritime traffic, on the colonial ascidian *Botryllus schlosseri* (Pallas 1766).

Methods

We performed two kind of noise treatments (duration: 30 min; 63-125 Hz; sound pressure level 160dB), using 1) a continuous noise and 2) an intermittent noise. To evaluate the noise effects, we used behavioral tests. The siphon stimulation test involves the oral siphon receptors, whose stimulation induces the oral siphon contraction. The tentacle stimulation test regards the coronal cells that, when stimulated, evoke the atrial siphon closure. The two tests evaluated the sensory cell ability to respond to a controlled mechanical stimulation before and after the noise treatments. Each test was accompanied by heartbeat counting to assess the potential effects also at physiological level. Treatments were performed in triplicates; untreated colonies (genetically identical to treated colonies) were used as control. Data were statistically analyzed.

Results

The continuous stimulation had negative effects at both behavioral and physiological level, significantly decreasing animal sensitivity and decreasing the heartbeat frequency; *vice versa*, colonies showed no effects when treated with intermittent noise.

Main conclusion

B. schlosseri is affected by the applied continuous noise. To improve our conservation policies, future experiments will be performed to verify if there is a threshold level negatively affecting these animals.

Macroevolution

Diving into the Past: Exploring Ascidian Biodiversity through Historical and recent records

Emanuela De Lisa[1], **Loriano Ballarin**[1], **Jacopo Bernardi**[1], **Giacomo Sabbadin**[1], **Federico La Torre**[1], **Lucia Manni**[1]

[1] Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35131 Padova. Emanuela De Lisa: emanuela.delisa@phd.unipd.it; Loriano Ballarin: loriano.ballarin@unipd.it; Jacopo Bernardi: jacopo.bernardi94@gmail.com; Giacomo Sabbadin: giacomo.sabbadin.1@studenti.unipd.it; Federico La Torre: federico.latorre@studenti.unipd.it; Lucia Manni: lucia.manni@unipd.it.

Aim

The Venice lagoon is a delicate environment where Non-Indigenous Species (NIS) are abundant and human impact is high. This work represents a literature-based study of the ascidian biodiversity (Tunicata, Ascidiacea) in the lagoon, considering records from the eighteenth century. Although ascidians are the most abundant taxon at the climax of the ecological succession on hard substrata and some have a strong invasive potential, the knowledge on their biodiversity is fragmented.

Methods

We used books provided by the Natural History Museums of Venice and Trieste, collection catalogues belonging to the Natural History Museums of Chioggia, Padua and Venice, and public online databases. We considered species lists, drawings, and descriptions. Species names were checked using the WORMS platform.

Results

We created a timeline from 1700 up to today, finding that, in the Venice lagoon, the first ascidian description dates to Stefano Chiereghin in 1784. Giuseppe Olivi's Zoologia Adriatica (1792) described *Alcynium conicum* and *Alcyonium schlosseri*, now known as *Aplidium conicum* and *Botryllus schlosseri*, respectively. However, the last comprehensive study was published by Brunetti in 1977, reporting five solitary and seven colonial species. Notably, *Perophora multiclathrata* and *Styela plicata* were later classified as NIS. Other NIS, including *Botrylloides violaceus*, *Didemnum vexillum*, and *Styela clava*, were later identified.

Moreover, ascidian sampling contributed to solve controversial taxonomical issues, allowing the description of *Ciona robusta* and *Ciona intestinalis* (previously considered a single species) and of *Botryllus schlosseri*. Additionally, a new cryptic species, *B. gaiae* was described within *B. schlosseri* complex.

Main conclusion

Despite in the Lagoon of Venice ascidians are known since the eighteenth century, information on their biodiversity is fragmented. Therefore, in consideration of their ecological role, an urgent action is required for their monitoring, according to the PNRR aims.

Macroevolution

Developing *C. robusta* juveniles as an experimental system in comparative immunology of invertebrate chordates

Assunta Liberti[1], Carla Pollastro[1], Gabriella Pinto[2,3], Anna Illiano[2,3], Rita Marino[1], Angela Amoresano[2,3], Antonietta Spagnuolo[1], Paolo Sordino[4]

[1] Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Naples, Italy. assusy.liberti@gmail.com, carla.pollastro9@gmail.com, rita.marino@szn.it, antonietta.spagnuolo@szn.it.
[2] Department of Chemical Sciences, University of Naples Federico II, Via Cinthia 26, 80126 Naples, Italy. gabriella.pinto@unina.it, anna.illiano@unina.it, angamor@unina.it.
[3] Istituto Nazionale Biostrutture e Biosistemi-Consorzio Interuniversitario, Viale delle Medaglie d'Oro, 305, 00136 Rome, Italy.
[4] Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Messina, Italy.

Aim

The study of inflammatory mechanisms is essential to understanding the role of the immune system in the interaction with microbiota. The metamorphic stage 4 of juvenile development is the first filter-feeding stage in the life cycle of the tunicate ascidian *Ciona robusta*. This stage can be used to explore *in vivo* the activation of key components of the innate immune response in the whole animal after exposure to different pathogen-associated molecular patterns (PAMPs) at the early phase of immune system development and maturation.

Methods

Transcriptional and targeted proteomic approaches were used to investigate the inflammatory response induced by exposure to stimuli of bacterial (lipopolysaccharide -LPS-, diacylated lipopeptide -Pam2CSK4) and fungal (zymosan).

Results

Our findings show an interconnection between different pathogen recognition receptors pathways and indicate the upregulation of cytokine gene expression as markers of inflammation. A first protein-protein interaction map was constructed to predict potential molecular interactions, and to correlate changes observed at transcriptional and translational levels.

Main conclusion

We gathered evidence in support of specific transcriptional and translational changes induced by PAMPs, and of possible interactions between molecules and pathways involved in the immune response, specifically TLRs and Dectin-1 pathways. In summary, this study depicts *C. robusta* as a unique chordate organism for studying factors and mechanisms that modulate immune activation and homeostasis, thus supporting its use as a viable experimental system in translational research and biotechnological approaches.

Macroevolution

Exploring the *Ciona robusta* immunome

Carla Pollastro[1,3], Assunta Liberti[1], Antonietta Spagnuolo[1], Paolo Sordino[2]

[1] Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Naples, Italy.
carla.pollastro9@gmail.com, assusy.liberti@gmail.com, antonietta.spagnuolo@szn.it.
[2] Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Messina, Italy.
paolo.sordino@szn.it.
[3] Tigem, Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, Pozzuoli (Naples), Italy.

Aim

To expand our knowledge of the immunogenomic landscape in chordates, we widened the spectrum of molecules that could be responsive to inflammatory stimuli in tunicate ascidians, the closest relatives of vertebrates. A homology search analysis of the tunicate ascidian *Ciona robusta* genome and proteome was performed by focusing on potentially immunocompetent molecules belonging to, or strictly correlated with, JAK-STAT signaling, Immunoglobulin and Dectin pathways.

Methods

A structure-, function- and conservation-based bioinformatics approach was used to identify orthologs of human immune-response genes and proteins. Our study has been conducted using specific databases and bioinformatic tools (e.g., BLAST, InterPRO, Cytoscape, iTASSER-MTD, Jalview) to identify a list of molecules, whose expression has been validated in *C. robusta* juvenile stage. Action mechanism of such molecules has been predicted through analysis of protein functional domains and gene regulatory elements, protein-protein interactomes and phylogenetic trees to better understand their evolutionary history.

Results

In a comparative approach to human immune-related pathways, the homology approach allowed to identify a high number of Gene Ontology terms, highly interconnected, corresponding to almost 50 uncharacterized genes containing CLECT, IRF and Ig domains. Several of these molecules are barely or absolutely uncharacterized in *C. robusta*. The immune-related activity of 11 immune-competent genes has been newly described and confirmed after inflammatory challenges. Finally, a protein-protein interactome network was built with the aim to identify the connections between molecular pathways involved in the immune response.

Main conclusion

Trying to gain a wider view of the complex evolutionary puzzle at the intersection between invertebrates and vertebrates, our findings indicate that the number of immune-related genes in ascidians is higher than previously known. This study depicts *C. robusta* as a unique organism to gain insights into the evolution of the immunome.

Macroevolution

DEEPOLY: Combining different molecular approaches to study deep-sea polychaetes in the Mediterranean

Sara Verni[1,2], Joachim Langeneck[3], Sergio Stefanni[2], Luigi Musco[2,3,4], Gabriele Cananzi[1], Alberto Castelli[1]

[1] Department of Biology, University of Pisa.
[2] Stazione Zoologica Anton Dohrn, Napoli.
[3] Consorzio Interuniversitario per le Scienze del Mare, CoNISMa, U.L.R. di Lecce.
[4] Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce.

Aim

Deep-sea ecosystems are among the biosphere's most extensive biomes, and polychaetes are the most abundant benthic invertebrates in deep-sea samples. Despite their important ecological role, and although the Mediterranean Sea is one of the most studied marine areas, the deep sea and its benthic macro- and meiofauna remain almost unexplored so far. To fill this gap, we are building a reference library of highly taxonomically accurate sequences for polychaetes from the deep Western Mediterranean Sea. We also aim to use this library in the study of environmental DNA obtained from the same study areas.

Methods

Two mitochondrial markers (16S rDNA and COI) were used to genotype 170 samples. Sequences were deposited in the Barcode Of Life Data System (BOLD); phylogenetic analyses were performed using maximum likelihood topologies with IQtree.

Environmental DNA was extracted from 58 sediment samples of the Palinuro and Marsili Seamounts, Canyon Dohrn and the Salerno epibathyal plain using the DNeasy Powermax Soil Kit, to perform further analyses on the composition of the polychaete community through metabarcoding.

Results

During the construction of the BOLD library, in many cases the specimens did not match any available sequence, thus representing the first data deposited for their lineage. Phylogeographic analyses revealed that deep-sea polychaetes from the Western Mediterranean include both Lusitanian and Senegalese species, as well as potentially endemic species, confirming the composite biogeographical origin of the Mediterranean biota also in deep-sea environments.

Main conclusion

The description of polychaete assemblages at the finest possible taxonomic level with integrative taxonomy and the subsequent use of eDNA will contribute to a more accurate assessment of their diversity and rate of endemicity. This is needed also to obtain reliable estimates of the effect of anthropogenic drivers of change on this poorly studied ecosystem.

Population & Species

Microbiota associated with meiofaunal nemerteans reveals evidence of phylosymbiosis

Francesca Leasi[1], Ester M. Eckert[2], Jon L Norenburg[3], W Kelley Thomas[4], Joseph L Sevigny[4], Jeffrey A Hall[4], Herman H Wirshing[3], Diego Fontaneto[2]

[1] Department of Biology, Geology, and Environmental Science, University of Tennessee at Chattanooga, Chattanooga, TN 37403, USA.
[2] National Research Council of Italy (CNR), Water Research Institute (IRSA), Molecular Ecology Group (MEG), Largo Tonolli 50, 28922 Verbania Pallanza, Italy.
[3] National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0163, USA.
[4] Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03823, USA.

Abstract

Phylosymbiosis is the association between the phylogenetic relatedness of hosts and the composition of their microbial communities. This phenomenon is widespread in diverse animal taxa. However, the generality of the existence of such a pattern has been questioned, and there seems to be evidence against its occurrence in small-sized aquatic animals, for which the microbiota composition is mostly shaped by local environmental factors rather than the hosts' phylogenetic relationships. This study aims to investigate the microbial communities associated with poorly known marine interstitial nemerteans to uncover their microbiota diversity and assess the occurrence of phylosymbiosis. Through a comprehensive approach, specimens from various Central American sites were analyzed using morphology-based taxonomy and molecular techniques targeting the host 18S rRNA gene, while their microbial associations were analyzed by targeting the prokaryotic 16S rRNA gene. Phylogenetic and statistical analyses were conducted to examine the potential effects of host nemertean taxa and sampling locations on the host-associated microbial communities. The results provide compelling evidence of phylosymbiosis in meiofaunal nemerteans, emphasizing the significant impact of host genetic relatedness on microbiome diversity, even in small-sized animals. Additional research is needed to fully unravel potential symbiotic relationships, as well as the complex mechanisms that govern the relationships between hosts and their microbiota across various organisms and ecological settings. The remarkable diversity of meiofaunal animals, spanning various animal phyla with different lifestyles and inhabiting diverse ecosystems, combined with advancements in multi-omics approaches, offers a promising avenue for a comprehensive understanding of the evolutionary and ecological interactions between hosts and their microbiota throughout the animal tree of life.

Population & Species

Structural and functional ecological traits in *Gongolaria barbata* individuals from a transitional water system

Maria Luisa Pica[1], Rosa Donadio[1], Ermenegilda Vitale[1], Giulia Costanzo[1], Marco Munari[2], Carmen Arena[1,3]

[1] Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy.
[2] Department of Biology, University of Padua, via U. Bassi 58/b, 35121 Padua, Italy.
[3] NBFC-National Biodiversity Future Center, Italy.

Aim

The ecological adaptation among individuals of different ages of habitat-forming species, i.e., *Gongolaria barbata*, needs to be better investigated. Filling this knowledge gap could lead to a better prediction of how climate change will affect these ecosystems and their ecological processes. To pursue this aim, individuals of different ages of *G. barbata* collected from the Venice Lagoon (Venice, Italy) were analysed for some peculiar structural and functional traits and put in relationship with their growth environment and ecological variables experienced during their development.

Methods

In vivo chlorophyll fluorescence emission analysis was performed on adult and young thalli of *G. barbata,* utilizing a pulse amplitude-modulated fluorometer (Junior-PAM, Walz Gmbh, Effeltrich, Germany). On samples, the expression of the D1 protein of photosystem II, photosynthetic pigments, and antioxidant activity was also determined to study the adaptive response of *G. barbata* thalli of different ages from the Venice Lagoon.

Results

The overall analyses showed increased thermal energy dissipation processes in adults with high pigment concentration and dry matter content; conversely, young individuals exhibited a higher photosynthetic yield, electron transport rate, and an elevated antioxidant level.

Furthermore, no difference in maximum photochemical efficiency and Photosystem II D1 protein was found between thalli of different ages.

Main conclusion

Our analysis highlighted remarkable differences between thalli of diverse age in *G. barbata*: the youngers allocate more energy into chemical defences and are focused on growth, whereas adults are more concerned about photoprotection and may have adapted over time, adjusting the photosynthetic rate, and opportunely modulating pigments and antioxidants concentration.

Population & Species

Impact of micro and nanoplastics on the gills, digestive glands, and byssus apparatus in *Mytilus galloprovincialis*

Rossana Romano[1,2], **Luigi Rosati**[3], **Palma Simoniello**[1,2]

[1] Department of Science and Technology, University Parthenope, Centro Direzionale, Is. C4, 80143 Naples, Italy.
[2] International PhD Programme UNESCO Chair "Environment, Resources and Sustainable Development", Villa Doria d'Angri, via F. Petrarca n. 80 (80123), Naples, Italy.
[3] Department of Biology, University Federico II of Napoli. Complesso Universitario Monte Sant'Angelo – Ed.7, Via Cinthia 26, 80126 Naples, Italy.

Aim

Microplastics (MPs) and Nanoplastics (NPs) pollution has become a global environmental problem due to their abundance, poor biodegradability, toxicological properties, and negative effects on aquatic and terrestrial organisms including humans (Anik et al., 2021). In our study, we investigated the cytotoxic effects of MPs and NPs in *Mytilus galloprovincialis*, a good bioindicator of marine pollution (Curpan et al., 2022).

Methods

We detected the effects of polystyrene on the fitness of mussels, analysing gills, digestive glands and byssus apparatus respectively involved in the respiratory, feeding process and adhesion to the substrate. Animals were exposed to polystyrene (5 or 0.1 µm) for 1, 3 and 11 days at environmental concentrations (Vroom et al., 2017). Samples were processed for light microscopy using different specific histological (Haematoxylin-eosin, Mallory's Trichrome, PAS, Alcian Blu and Picrosirius Red) or Immunohistochemistry staining.

Results

Histological analysis demonstrated that MPs and NPs are cytotoxic for mussels interfering with structure of gills tissues. Disorganization on the septum and lamellar structures were observed. Melanin and lipofuscin infiltrations were detected, alteration in numbers distribution of mucus cells after treatments also were observed. Immunocytochemistry investigations by PCNA demonstrated an increasing number of positive cells to the antibody confirming alterations in cell cycle. HSP70 detection indicated marked cellular stress response. MPs and NPs affect the digestive gland structures in the tubules and ducts organization, interfering with the proliferative activity, inducing collagen deposition within the digestive tubules. Alterations in number and organization of mucus cells after short and long-time exposure were observed. Impairment in the byssus glands structures and altered byssal thread production were detected.

Main conclusion

Polystyrene induces damage in gills, digestive glands and byssus apparatus of mussels, altering their functions, this could represent a risk for their health, for the food chain and indirectly for human consumers.

Population & Species

I came along with you: first Mediterranean record of a Red Sea nudibranch, *Lomanotus vermiformis* Eliot, 1908, three decades after its favourite hydrozoan prey, *Macrorhynchia philippina*

Yann Toso[1], Andrea Toso[1], Lara Marastella Fumarola[1], Marcella D'Elia[2], Michel Bariche[3], Stefano Piraino[1,4,5], Giulia Furfaro[1,4,5]

[1] Department Biological and Environmental Sciences and Technologies-DiSTeBA, University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. yann.toso@unisalento.it; andrea.toso@unisalento.it; laramarastella.fumarola@unisalento.it; stefano.piraino@unisalento.it; giulia.furfaro@unisalento.it.
[2] Department of Mathematics and Physics "Ennio De Giorgi", University of Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy. marcella.delia@unisalento.it.
[3] Department of Biology, Faculty of Arts and Sciences, American University of Beirut, PO Box 11-0236 Beirut, Lebanon. Mb39@aub.edu.lb.
[4] Research Unit Lecce, Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), 73100 Lecce, Italy.
[5] National Biodiversity Future Center (NBFC), 90133 Palermo, Italy.

Aim

Lomanotus vermiformis is a dendronotid nudibranch (Gastropoda, Heterobranchia) with circumtropical distribution. It is exclusively reported on the hydroid *Macrorhynchia philippina* Kirchenpauer, 1872, where it lives undisturbed due to its body pattern perfectly resembling the shape of the colony. In fact, this nudibranch is adapted to this powerful stinging hydrozoan on which it is supposed to feed and lay its eggs. Originally reported from the Red Sea, the scanty description was followed by a more detailed one based on specimens from Australia, a location far from its type locality. Considering that *M. philippina* has recently colonized the Mediterranean Levantine basin, the possible co-occurrence of its associated nudibranch was investigated and confirmed for the first time.

Methods

Within the framework of the project *BLUE TYRE – Local Partnership for Sustainable Marine and Coastal Development* (AID 012314/01/6), five branches of *M. philippina* were collected while scuba diving in Tyre (Lebanon) and sorted in laboratory. Morphological analysis was carried out using light and SEM microscopy on the nudibranch, its gut content, and the hydroid.

Results

Six *L. vermiformis* were identified, confirming the presence of the species in the Mediterranean Sea. An updated redescription of the diagnostic features (buccal apparatus and reproductive system) is provided based on specimens coming from the type locality and Lessepsian in the Mediterranean basin. Furthermore, the trophic relationship between the hydroid and its host was demonstrated through analyses of the nudibranch's radula and gut content, revealing, for the first time the presence of microbasic mastigophore nematocysts that match those observed in the nematothecae of *M. philippina*.

Main conclusion

The present finding emphasizes the importance of monitoring Mediterranean alien structural tridimensional species to early detection of associated non-indigenous fauna. Moreover, new insights are provided on this strong adaptive association, shedding lights on the poorly known biology characterizing mimetic and elusive nudibranch species.

Thanks to all the participants!