

Book of Abstracts

EVOLMAR 2020

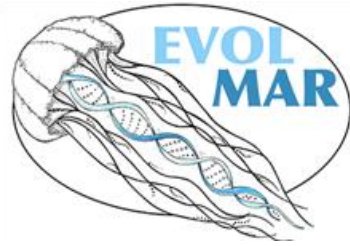
Marine Evolution 1st Italian Congress

online
23-25 November 2020



Stazione
Zoologica
Anton Dohrn
Napoli





Book of Abstracts

23-25 November 2020



Organizing Committee

Maria Vittoria Modica - *Chair*

Stazione Zoologica Anton Dohrn, Napoli (Italy)

Giulia Furfaro, Università del Salento, Lecce (Italy)

Donato Giovannelli, Università Federico II, Napoli (Italy)

Emiliano Mancini, Sapienza Università di Roma (Italy)

Marco Munari, Stazione Zoologica Anton Dohrn, Napoli (Italy)

Francesco Santini, GE.B.I. (Italy)

Sergio Stefanni, Stazione Zoologica Anton Dohrn, Napoli (Italy)

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

Scientific Committee

Lisa Locatello - *Chair*

Stazione Zoologica Anton Dohrn, Napoli (Italy)
Università di Padova (Italy)

Chiara Benvenuto, Salford University (UK)

Ilaria Coscia, Salford University (UK)

Roberto Feuda, Leicester University (UK)

Marco Gerdol, Università di Trieste (Italy)

Francesca Leasi, University of Tennessee Chattanooga (USA)

Luca Mirimin, Galway-Mayo Institute of Technology (Ireland)

Paolo Sordino, Stazione Zoologica Anton Dohrn, Napoli (Italy)

Web Design: **Giuseppe d'Errico**

Logo: **Daniela Ronchetti**

Photo credits: **Susanne Bähr** (photo title: *Symbiotic Hitchhiker*)

Sponsors



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

The First Italian Conference on Marine Evolution - Primo Congresso Italiano di Evoluzione Marina (EVOLMAR 2020) took place in a virtual format on November 23rd-25th, 2020. The conference was organized by the Stazione Zoologica Anton Dohrn, Naples (SZN), the Dipartimento di Biologia e Biotechnologie Charles Darwin, Sapienza University, Rome, and the Italian Society for Evolutionary Biology (SIBE-ISEB), in addition to a number of supporting sponsors.

The meeting featured a combination of invited keynotes, contributed talks and posters, for a total of 92 presentations arranged in a program revolving around 4 thematic areas: macroevolution, populations and species, adaptation, biodiversity. All abstracts submitted are included in this book. Some of the abstracts were edited to conform to the meeting format, however abstract content was not modified and is that originally submitted by the authors. The name of the presenting author, when multiple authors are present, is underlined.

KEYNOTE SPEAKERS

Giacomo Bernardi, University of California Santa Cruz

Fiorenza Micheli, Stanford University

Maria Pia Miglietta, University of Texas A&M

Davide Pisani, University of Bristol

Red Sea fishes in the Mediterranean: the genomics of Lessepsian bioinvasions

*Giacomo Bernardi**

* Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA, USA

Aim

The opening of the Suez Canal, in 1869, connected the Red Sea and the Mediterranean, involuntarily creating a grand experiment in biological invasion science. At first, few species migrated from the Red Sea into the Mediterranean (termed Lessepsian bioinvaders), yet the rate of invasion kept increasing with time. While the expectation of few individual invaders should result in signatures of genetic bottlenecks, this is seldom observed. The goal of our studies is to identify the genetic patterns of Lessepsian bioinvaders, and determine the role of natural selection in the invasion success.

Methods

A combination of classical Sanger sequencing and genomic RAD sequencing was used on a panel of fish species. Samples were obtained for both early invaders and more recent ones, as well as from sites that are very close to the Suez Canal as well as far from it.

Results

For the most part, we have found that successful invaders do not show a signature of bottlenecks, instead, their genetic diversity is similar to what is seen in natural Red Sea populations. Blue spotted cornetfish, in contrast, shows reduced genetic diversity and evidence for genes under selection, in particular for genes that are related to osmoregulation, a key factor in being able to breach the barrier of the Canal.

Main Conclusion

While most Lessepsian migrants do not show evidence of bottlenecks and founder effects, a combination of population size and local adaptation seem to play essential roles in successful invasions.

Thematic Areas

Macroevolution, Populations and Species

Insights into cellular reprogramming from the immortal jellyfish *Turritopsis dohrnii*

*Maria Pia Miglietta**, *Yui Matsumoto**

Texas A&M University at Galveston, Galveston, TX, USA

When faced with stressful conditions, such as lack of food or physical damage, the medusae of *Turritopsis dohrnii* avoid death by reverting to the polyp stage, passing through a short-lived cyst stage. In the cyst stage, cellular transdifferentiation occurs. Because of its unique potential for rejuvenation, and because this life cycle reversal can be induced under controlled laboratory conditions and in 24-48 hours, *T. dohrnii* represents an unparalleled model system to investigate the molecular mechanisms that control stage-specific cell differentiation. We present the assembled transcriptome of the *T. dohrnii* life cycle stages. We perform time-series differential gene expression analyses to identify genes enriched in the cyst during the reverse developmental sequence (i.e., from wild polyp to medusa, cyst, and reversed polyp). Using pairwise differential gene expression analyses, we compare the benthic polyp and planktonic medusa's expression profiles and those of the polyps generated by different developmental pathways (colonial polyp generated by asexual budding, and polyp generated from the cyst through reverse development). We conduct a preliminary screening of our RNA-seq libraries and transcriptome assembly to test whether homologs of the Yamanaka and Thompson transcription factors (Oct4, Sox2, Klf4, c-Myc, Nanog) that govern the induction of pluripotency in humans are present in *T. dohrnii*. Finally, we investigate whether Sirtuin proteins, telomerase and regulators of telomere elongations, heat shock proteins, Yamanaka transcription factors, and proteins in the AMPK signaling pathway, all canonically involved in processes of tissue regeneration, cell plasticity, and aging in vertebrates and Metazoa, play a role in *T. dohrnii*'s reverse development.

Ocean Solutions for Nature and People

Fiorenza Micheli

Hopkins Marine Station and Stanford Center for Ocean Solutions, Stanford University, Pacific Grove, CA 93950, USA.

The ocean is Earth's last frontier. It comprises most of its habitable volume, is home to a unique and extraordinary diversity of plants, animals, and microbes; regulates its climate; and provides food and livelihood for billions of people. Our future is inextricably linked to the ocean, and to maintaining the flow of critical and irreplaceable services healthy oceans provide. While much investigation and discussion is focused on impacts, there is a critical need and opportunity to develop solutions based in oceans. I will present and discuss ocean-based solutions to two grand challenges, climate and food and nutritional security, highlighting knowns and unknowns in their potential for deployment at scale, and opportunities for expanding global capacity for ocean solutions.

Can the relationships at the root of the animal tree be solved?

Davide Pisani¹, Gert Wörheide², Roberto Feuda³, Ksenia Juravel², & Maria Eleonora Rossi¹.

¹ School of Earth Sciences and School of Biological Sciences, University of Bristol.

² Dept. of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München

³ Department of Genetics, University of Leicester

Aim

Understanding relationships at the root of the animal tree is key to clarify animal evolution and the origin of modern marine ecosystems. Traditionally the sponges were seen as the sister group to all the other animals and the last common ancestor of all animals was assumed to be a sponge-like organism. However, standard analyses of amino acid data failed to provide a consistent support for this hypothesis, frequently finding support for Ctenophora (comb jellies), as the sister group of all the other animals instead (the Ctenophora-sister hypothesis). I will summarise current evidence and recent advances bearing on the topic and discuss how they impact our understanding of early animal evolution.

Methods

A diversity of published amino acid data sets were analysed using a multiple models and data transformations, in a Bayesian context, to evaluate changes in support, fit and adequacy. Morphological datasets including both fossils and extant taxa were analysed using Bayesian and other methods and presence/absence of individual orthogroups and protein families were analysed using Bayesian methods.

Results

A clear pattern of consistency exists and different data types (Genome content, Morphology and Amino Acid data analysed using well-fitting models) can be shown to discriminate against the Ctenophora-sister hypothesis. Amino acid data analyses are more variable and strongly model-dependent; implying that model selection and model adequacy should be the guiding principle when amino acid data are used.

Main Conclusion

While there is still significant uncertainty on the relationships at the root of the animal tree, the Porifera-sister hypothesis is supported by multiple lines of evidence. While several amino acid models support Ctenophora-sister, support for this hypothesis is invariably maximised under the worst fitting models.

Thematic Areas

Macroevolution, Biodiversity



CONTRIBUTED PAPERS

Genetic structuring in endangered yellow-nosed albatross species complex

Dilini Abeyrama¹, Zach Dempsey¹, Peter G. Ryan², Theresa M. Burg¹

¹University of Lethbridge, Lethbridge, Alberta, Canada

²FitzPatrick Institute of African Ornithology, DST-NRF Centre of Excellence, University of Cape Town, Rondebosch 7701, South Africa

Aim

The two species of yellow-nosed albatross, Atlantic (*Thalassarche chlororhynchos*) and Indian (*Thalassarche carteri*), are morphologically similar, however, they show some differences in behaviour and breeding range.

Methods

We studied genetic variation within and among the two species using nuclear (microsatellite, Pema7 and Occa9) and mitochondrial (control region) markers. We analysed 354 samples from four breeding islands and two bycatch locations (Atlantic: Nightingale, Inaccessible and Gough; Indian: Amsterdam Island, and bycatch samples from South African and New Zealand).

Results

Both sets of markers clearly differentiated the two species. Microsatellite and Occa9 nuclear markers revealed two genetically distinct groups within Atlantic yellow-nosed albatross, separating birds from Nightingale and Inaccessible Islands from those on Gough Island. All of the South African and New Zealand bycatch samples were Indian yellow-nosed albatross, but genetically distinct from the Amsterdam Island birds and from each other. Both species have distinct at-sea distributions. Within both species differences in at-sea distribution might also have resulted in genetic differentiation, but there is currently no evidence of intraspecific differences.

Main conclusion

The two yellow-nosed albatross species are listed as endangered due to their limited number of breeding sites and threats from introduced diseases, introduced predators and fishing mortality. As such, our results contribute to conservation and management and suggest the need for separate management of genetically distinct groups of Atlantic and Indian yellow-nosed albatrosses breeding at different islands.

Thematic Areas

Populations and Species

Naso-oral region analysis reveals a phylogenetic pressure in the Houndshark

Aicardi Stefano, Longo Alessio, Gallus Lorenzo, Ferrando Sara

Department of earth, environmental and life sciences (DISTAV), University of Genoa, Italy

Aim

The shape of the naso-oral region in elasmobranch species changes from elongated and thin to short and flat. That shape may be related to the phylogeny, environmental factors, types of habitat and feeding strategies. In the frame of sensory biology, the shape of the head is related to the surface area for the distribution of the electroreceptors and affects the water flow on the olfactory chamber.

Methods

Geometric morphometrics is a quantitative analysis useful to compare morphology considering homologous points called landmarks.

Results

The data distribution on the multidimensional space through principal component analysis shows the differences responding to environmental or evolutionary pressures. The Triakid is a heterogeneous family, it belongs to the Carcharhiniformes and counts six genera with both benthonic and pelagic deep species, both coastal and oceanic species, both squid-feeder only and generalist species. The preliminary study presented here proposes the analysis of the Triakid's naso-oral region considering rostrum, mouth, nares, and nasal flaps as landmarks. The naso-oral shape of 42 out of at least 45 Triakids species is plotted on a two dimensions plane, which describes the 53% of shape variance. Moreover, the result is coupled with a phylogenetic tree based on the mitochondrial gene of NADPH2 and shows a different species' clusters, apparently determined by phylogeny rather than ecological traits.

Main conclusion

This preliminary work shows that in a numerous sharks' family, the main morphological differences in naso-oral region are produced by the phylogenetic relationship. More investigations will be necessary to understand the changes in shape all over the taxonomic levels in elasmobranch species.

Thematic Areas

Adaptation

An endocrine signaling integrates energy homeostasis and lunar phase to regulate aspects of growth and sexual maturation in *Platynereis*

Gabriele Andreatta^a, Caroline Broyart^{a,2}, Charline Borghgraef^b, Karim Vadiwala^a, Vitaly Kozin^{a,3}, Alessandra Polo^{a,4}, Andrea Bileck^c, Isabel Beets^b, Liliane Schoofs^b, Christopher Gerner^c, Florian Raible^a

^a Max Perutz Labs, University of Vienna, A-1030 Vienna, Austria

^b Animal Physiology and Neurobiology, Department of Biology, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

^c Department of Analytical Chemistry, University of Vienna, A-1090 Vienna, Austria

² Present address: The Plant Signaling Mechanisms Laboratory, Department of Plant Molecular Biology, University of Lausanne, 1015 Lausanne, Switzerland

³ Present address: Department of Embryology, St. Petersburg State University, St. Petersburg 199034, Russia

⁴ Present address: Reef Renewal Foundation Bonaire, Kralendijk, Bonaire, Caribbean Netherlands

Aim

The molecular mechanisms by which animals integrate external stimuli with internal energy balance to regulate major developmental and reproductive events still remain enigmatic. In vertebrates, this coordination is regulated by gonadotropin-releasing hormone (GnRH), which plays a crucial role in sexual maturation and reproduction. Whether this regulation is conserved in invertebrate species is still unknown.

Methods

We combined extensive phenotypic analysis with gene expression studies and proteomics to shed light on the role of this endocrine signalling in the marine bristleworm, *Platynereis dumerilii*, a species where sexual maturation is tightly regulated by both metabolic state and lunar cycle.

Results

We find *gnrh-like* (*gnrhl*) genes upregulated in sexually mature animals, after feeding, and in specific lunar phases. Animals in which the *corazonin1/gnrhl1* gene has been disabled exhibit delays in growth, regeneration, and maturation. Molecular analyses reveal glycoprotein turnover/energy homeostasis as targets of CRZ1/GnRHL1.

Main conclusion

Taken together, these data shed light on the endocrine mechanisms coordinating the energy demands dictated by environmental and developmental cues, and how they contribute to time reproductive events.

Thematic areas

Populations and Species, Biodiversity

Multidrug-resistant marine bacteria isolated from the shallow hydrothermal vent systems of Panarea (Aeolian Islands)

*Erika Arcadi^a, Monique Mancuso^{ab}, Marilena Sanfilippo^a, Danilo Malara^a, Franco Andaloro^a,
Valentina Esposito^c, Cinzia De Vittor^c, Teresa Romeo^a*

^aStazione Zoologica Anton Dohrn, Centro interdipartimentale della Sicilia, Messina, Italy

^bInstitute for Marine Biological Resources and Biotechnology (IRBIM), National Research Country (CNR), Messina, Spianata S. Raineri 86, 98122 Messina, Italy

^cIstituto Nazionale di Oceanografia e di Geofisica Sperimentale – OGS Borgo Grotta Gigante 42/C – 34010 Sgonico (TS) – Italy

Aim

In the present study was evaluated the antimicrobial resistance of marine heterotrophic bacteria isolated from the recently discovered shallow-hydrothermal vents of Panarea, located in the Volcano Archipelago of Aeolian Islands. After recording the environmental parameters, water and sediment enrichments were carried out from the Bottaro Crater (depth, 9 m)(CB) where the lowest pH values were recorded and from the Hot / Cold systems site, (depth,10-12 m)(HL and CL), this last site characterized by three hot and three cold plots.

Methods

Fifteen bacterial strains were isolated on Marine Agar from both water and sediment samples, that is five strains from each site (CB 1-5; HL 1-5; CL 1-5). Each one was tested against 17 antibiotics. The growth of the bacterial strains was tested with different salinities and temperatures.

Results

Results showed that the majority bacterial samples presented a multidrug resistance from 6 to 15 antibiotics. In particular the most resistant bacteria were isolated from Bottaro Crater and Hot lake. Among the antibiotics the highest resistance percentage (87%) was recorded for Tetracycline, Oxacillin and Clindamycin. Salinity test has showed that 50% is able to growth at 150g/L NaCl, but No strain showed any growth on 200g/L NaCl. Temperature test has showed that HL4, HL5,CL4 strains were able to growth at 4°C, but only HL5 and CB4 are able to growth at 50°C, being among all the strains analyzed, the most tolerant . All can grow between 15-40°C.

Main Conclusion

Our investigation might do understand if these strains analyzed have modified something in their genome by establishing whether the resistance to the tested antibiotics they have developed is related to their adaptation to these extreme environments, as demonstrated from previous studies. The species of these strains is not yet known, but over all we have obtained a good resistance response to antibiotic screening.

Thematic Areas

Adaptation, Biodiversity

Diversity and biogeography of reef corals across the Indo-Pacific based on genomic RAD data

Roberto Arrigoni¹, Michael L Berumen², Kiruthiga G Mariappan², Andrew H. Baird³, Danwei Huang⁴, Tullia Isotta Terraneo², Simone Montano⁵, Davide Maggioni⁵, Francesca Benzoni²

¹ Department of Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn Napoli, Villa Comunale, 80121, Naples, Italy

² Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia

³ ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland 4810, Australia

⁴ Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore

⁵ Department of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, Milano 20126, Italy

Aim

Accurate delimitation of species and their relationships is a fundamental question in evolutionary biology and systematics and provides essential implications for conservation and management. Scleractinian corals are difficult to identify because of their morphological plasticity. Furthermore, little is known about their distribution and biogeography. For example, many coral species are thought to be widely distributed in the Indo-Pacific but limited genomic information are available to verify this hypothesis.

Methods

Here, we applied the genome-wide technique Restriction-site Associated DNA sequencing (ezRAD) to investigate phylogenetic relationships and biogeography within two common reef coral genera of the Indo-Pacific, namely *Leptastrea* and *Cyphastrea*. We collected 180 colonies from several localities across the Indo-Pacific, from the Red Sea to French Polynesia, and subjected them to genomic analyses. A combination of de novo clustering and reference mapping was used to obtain coral and holobiont SNPs and to retrieve nearly complete coral mitochondrial genomes.

Results

Phylogenetic analyses reveal high diversity within the two genera, with some lineages in agreement with morphology-based taxonomy and several lineages representing undescribed species. Only a minor fraction of the genomic lineages occurs across the Indo-Pacific, displaying strong clustering and isolation by distance between the Indian and Pacific populations. Most lineages are found either in the Indian or Pacific basin, with the seas around the Arabian Peninsula as a region of high endemism.

Main conclusion

This study suggests that an important fraction of hard coral biodiversity is yet to be discovered and that actual coral distributions are narrowed than traditionally thought.

Thematic areas

Populations and Species, Biodiversity

A comparison of metabolic and behavioural responses in sea urchins from different environments during long-term exposure to seawater acidification

Davide Asnicar¹, Marco Munari², Maria Gabriella Marin¹

¹ Department of Biology, University of Padova, 35121, Padova, Italy;

² Department of Integrative Marine Ecology, Ischia Marine Centre, Stazione Zoologica Anton Dohrn, Punta San Pietro, 80077, Ischia, (Naples), Italy.

Aim

From the beginning of the industrial revolution, about 40% of anthropogenic atmospheric CO₂ has been absorbed by the oceans causing a phenomenon called ocean acidification (OA). Calcifier organisms are the most sensitive to hypercapnia, reduction of pH and carbonate saturation, which are consequences of OA. Previous researches often showed contrasting results concerning OA, even within the same species. This could be related to differences in methods used and different sensitivity to environmental perturbations among local populations.

Our project aimed to assess the long-term effects of a predicted OA scenario on *P. lividus* adults, highlighting potential different plasticity in specimens from different locations.

Methods

Two experimental groups of sea urchins with a different ecological history were used. One was sampled in the Venice lagoon, with high environmental variability and anthropogenic disturbance. The other was sampled in a site far from freshwater inputs and human impacts in the Gulf of Trieste. Animals were maintained for six months in both natural (pH 8.1) and future predicted condition (pH 7.7). Monthly, physiological (respiration rate and ammonia excretion) and behavioural (righting and sheltering seeking) endpoints were investigated.

Results

Lagoon sea urchins' physiological endpoints were not influenced by pH. Contrarily, in coastal group ammonia excretion increased in low pH exposed animals.

In both lagoon and coastal sea urchins, fewer animals exposed to reduced pH performed righting and took longer to perform it. Moreover, coastal animals exposed to low pH moved less, slower and reached the shelter in smaller numbers.

Main conclusion

OA condition affected physiological and behavioural responses of animals from both collection sites. Although differences were observed between the two groups, under prolonged exposure animals were able to acclimate. Reduced pH affected the responses of the lagoon sea urchins less than those of their coastal counterparts. These findings suggest higher tolerance to environmental variations in lagoon specimens.

Thematic Areas

Adaptation, Populations and Species

Morphology and reproduction in the *Hapalocarcinus marsupialis* Stimpson, 1859 species complex (Decapoda: Brachyura: Cryptochiridae)

Susanne Bähr^{*}, *Magnus L. Johnson*[‡], *Sancia E.T. van der Meij*^{*§}

^{*}Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands

[‡]Department of Biological and Marine Sciences, University of Hull, Hull, United Kingdom

[§] Marine Biodiversity group, Naturalis Biodiversity Center, Leiden, The Netherlands

Aim

Gall crabs live in obligate symbiosis with tropical scleractinian corals and induce skeletal modifications in their hosts that are used as dwellings. Their unique biology has led to remarkable adaptations in morphology and reproductive output. However, due to their diminutive size (< 1 cm) and hidden lifestyle many aspects of their biology are still unknown. The gall crab *Hapalocarcinus marsupialis*, associated with Pocilloporidae corals, is thought to be a species complex based on genetic data, showing stricter host specificity than previously assumed. Here we use an integrative approach combining morphometrics and reproductive data to study host-adaptive speciation in *Hapalocarcinus*.

Methods

To study the diversity in *Hapalocarcinus*, and possible morphological and reproductive differences between putative species, we collected 163 specimens in the Red Sea from *Stylophora* and *Pocillopora* corals. Phylogenetic analyses were conducted using the barcoding gene COI. Carapace, abdomen and chelae measurements were taken using a stereomicroscope equipped with calibrated optical micrometer. Brood mass of ovigerous females was removed, fecundity was determined as number of eggs. Females and their eggs were dried and weighed to calculate reproductive output.

Results

Phylogenetic analyses revealed five candidate species in the *H. marsupialis* complex. Species a-b inhabit *Stylophora* corals, whereas species c-e inhabit *Pocillopora*. Candidate species differ in carapace, abdomen and chelae morphology. Moreover, crabs collected from *Pocillopora* are significantly bigger than those collected from *Stylophora*, and their fecundity is on average 117 % higher. The average reproductive output (69%) of *H. marsupialis* is high compared to non-symbiotic brachyurans (~20%).

Main conclusions

Morphometrics and reproductive traits are useful in separating the putative species in the *H. marsupialis* complex. We hypothesize that host specificity in *Hapalocarcinus* lead to morphological adaptations in the crabs, likely influenced by the size and morphology of their dwelling. Hence co-evolution is considered a driving factor behind species divergence.

Thematic Areas

Adaptation, Populations and Species

Sea turtle conservation: genetics and genomics for better management

Barbanti A^{1,2.}, Pascual M^{1.}, Carreras C^{1.}

1. Department of Genetics, Microbiology and Statistics and IRBio, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain.

2. Center for Advanced Studies of Blanes (CEAB-CSIC). C/ d'accés a la Cala St. Francesc, 14, 17300 Blanes, Girona, Spain.

Aim

Conservation actions aim to preserve and recover animal and plant species using *in-situ* or *ex-situ* strategies. Conservation genetics can provide important insights into the dynamics of endangered populations facilitating their management. This project shows how the use of traditional markers and new generation sequencing can benefit conservation management, focusing on the case studies of the loggerhead (*Caretta caretta*) and the green turtle (*Chelonia mydas*).

Methods

We used microsatellites and mtDNA to assess the outcome of a reintroduction program of green turtles in the Cayman Islands and the status of the reintroduced wild population. We performed parentage analysis between wild and captive population, tested for loss of genetic variability and assessed population structure. Then we explored the potential of 2b-RAD genomic sequencing, studying the population structure and local adaptation of 10 Eastern Mediterranean loggerhead turtle rookeries.

Results

In the Cayman Island case study, we found that 90% of adult wild females and 80% of wild F1 hatchlings were related to the captive population, proving the program successful. This relatedness did not affect negatively the fitness of the wild population. Moreover, we found that after only one generation, genetic differentiation between the populations was significant. In the Mediterranean case study, we found a high level of population structure and the PCoA showed three main groups of loggerhead turtle rookeries. Atmospheric temperature and geographic location of the rookery showed a significant impact on population structure, as outlier loci were found associated with these predictors. Finally, different effective population sizes denote that the studied populations belong to different classes of extinction risk.

Main Conclusion

Our results show the key role of genetics and genomics studies in conservation management. These analyses, in fact, can uncover details crucial for decision making in both in-situ and ex-situ conservation.

Thematic Areas

Populations and species

Negotiations Over Parental Care: A Test of Alternative Hypotheses in the Clown Anemonefish

T.A. Barbasch^{*}, R. Branconi^{*}, R. Francis^{*}, M. Pacaro^{*}, M. Srinivasan[†], G.P. Jones [†], P.M. Buston^{*}

^{*} Department of Biology and Marine Program, Boston University, 5 Cummington Mall, Boston MA, USA

[†] ARC Centre of Excellence for Coral Reef Studies, and College of Science and Engineering, James Cook University, Townsville, Queensland, Australia

Aim

In species with biparental care, conflict arises over how much each parent provides to their offspring because both benefit from shifting the burden of care to the other. Here, we tested alternative models for how parents negotiate offspring care using a wild population of clownfish (*Amphiprion percula*).

Methods

Using 60 breeding groups, we experimentally handicapped parents by fin-clipping the female in 20 groups, the male in 20 groups, and neither parent in 20 groups and measured changes in female, male, and pair combined effort in response to handicapping.

Results

First, we found that handicapping resulted in a decrease in the number of eggs laid by fin-clipped females and a decrease in the amount of parental care by fin-clipped males. Second, contrary to predictions, female effort did not change in response to the male being handicapped, or vice versa. Finally, the number of embryos that matured to hatching, an indicator of pair effort, was not influenced by the manipulation, suggesting that although the handicap was effective, clownfish do not face the predicted “cost to conflict” when one parent is handicapped.

Main conclusion

Together, these results question the generality of current theoretical predictions and expand our understanding of the diverse possible outcomes of parental conflict.

Thematic Areas

Adaptation, Populations and Species

How can EMODnet Biology be used for marine biodiversity studies?

*Joana Beja¹, Leen Vandepitte¹, Vasilis Gerovasileiou², Peter Herman³, Dan Lear⁴, Bart Vanhoorne¹,
EMODnet Biology partners*

1 Flanders Marine Institute, Belgium

2 Hellenic Centre for Marine Research, Greece

3 Deltares, The Netherlands

4 Marine Biological Association, United Kingdom

Aim

As a consequence of the 2007 European Union Maritime Policy, the European Marine Observation and Data Network (EMODnet) was established to improve access to high quality marine data. From 2009 to 2012, a set of preparatory actions led to the development of an online portal which allowed for the access and download of these data European marine biological data.

Methods

EMODnet Biology is currently a partnership between 22 organisations, but it has connections with other international initiatives such as OBIS and GBIF, ensuring that the data is not duplicated but can be widely accessed. We will show how the partnership is organised and the various ways data providers can submit their data and the different procedures we undertake to make data FAIR.

EMODnet Biology data includes and encourages the use of the World Register of Marine Species (WoRMS) for species scientific names, British Oceanographic Data Centre NVS2 for measurements or facts that accompany the data and Marine Regions for the sample locations, thus improving standardisation and interoperability with other data.

Results

Over the last 20 years, the European marine biological landscape has changed significantly. MarBEF included 94 marine institutes in its network and gave rise to EurOBIS and ERMS (which became WoRMS). With the setup of EMODnet Biology (meta)data interoperability and standardisation has been the main aim alongside the free and open access to the data.

EMODnet Biology currently provides access to almost 25 million records from over 1000 datasets of various taxonomic groups. It also provides access to an ever-growing portfolio of data products and various tools.

Main conclusion

EMODnet Biology data have been used in several assessments (UN World Ocean Assessments and IPBES regional and global assessments, both via OBIS) and have also contributed to an OSPAR assessment on the introduction of non-indigenous species.

Thematic Areas

Biodiversity and Population and Species

***Centrophorus* spp. and the mystery of the deep-sea: how many species in the Mediterranean?**

Anna Benvenuto¹, Andrea Bellodi², Riccardo Melis², Antonello Mulas², Alice Ferrari¹, Rita Cannas²,
Alessia Cariani¹, Maria Cristina Follesa²

¹Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Ravenna, Italy - anna.benvenuto@studio.unibo.it

² Department of Life and Environmental Sciences, University of Cagliari, Italy

Aim

The current shift of fisheries efforts towards deep seas is raising concern about the vulnerability of deep-water sharks which are often poorly studied and characterized by problematic taxonomy. In the Mediterranean Sea the taxonomy of genus *Centrophorus* (Müller & Henle, 1837) has not been clearly unravelled yet. Since a proper identification of species is fundamental for their correct assessment and management, in this study we try to clarify the taxonomy of this genus in the Mediterranean basin using integrated taxonomy technique to identify the species of the genus occurring in the basin.

Methods

281 samples were analysed obtaining sequences from three mitochondrial markers (COI, ND2 and 16S). All sequences were compared with those obtained from public databases (GenBank and BOLD) to delineate discrete taxonomic units and assess inter and intra-specific genetic diversity and variability. Molecular techniques were integrated with traditional and landmark-based morphometry to assess morphological variability and provide useful diagnostic characters for the discrimination of species.

Results

Molecular analyses show a low sequence variability for all the three markers analysed. A principal haplotype occurs through all the locations despite several private and shared haplotypes, excluding a clear geographical segregation. In phylogenetic trees the sequences cluster in a highly supported branch with other sequences deposited as *C. granulosus*, *C. uyato*, and *C. zeehaani*. From morphometric analyses, both traditional and landmarks-based, specimens cluster in a unique group representing a single morphotype.

Main conclusion

Both molecular and morphological results confirm the presence of a unique species of *Centrophorus* in the Mediterranean. Genetically, all specimens result in one clade ascribable to *C. cf uyato*. This finding is confirmed by morphological analyses that grouped all the specimens in a unique group representing a single morphotype.

Thematic Areas

Populations and Species, Biodiversity

Identification of microRNAs and piRNAs involved in the sea urchin *Paracentrotus lividus* germ line specification

*Ines Fournon Berodia*¹, *Francesca Rizzo*², *Konstaninos Geles*³, *Danila Voronov*¹, *Maria Ina Arnone*^{1*}

¹Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy

² Università Degli Studi di Salerno, Dipartimento di Medicina, Chirurgia e Odontoiatria “Scuola Medica Salernitana”/DIPMED, Via Salvador Allende, 43L, 84081 Baronissi SA

³University of Magna Graecia / Genomix4Life Srl, Laboratorio di Medicina Molecolare e Genomica, Campus di Medicina e Chirurgia, Prefab. 2 Via S. Allende, 1 - 84081 Baronissi (SA) – Italy

Aims

The aim of the project consists on the identification of microRNAs and piRNAs involved in sea urchin germ line specification. The species chosen for this project is *Paracentrotus lividus* and the cell type studied are the small micromeres (sMic). These cells are known to express germ line specific genes and contribute to the left coelomic pouch (LCP). The LCP grows and upon interaction with some cells invaginating from the adjacent ectoderm forms the rudiment that will then become the juvenile sea urchin after metamorphosis. Therefore, sMic descendant cells contribute to the sea urchin adult and contain germ line related genes.

Methods

To compile the smallRNA library, sMic enriched RNA is necessary and therefore the sMics are first isolated from the rest of the embryo via life cell labelling with Calcein-AM at the desired developmental stage, to then be single cell dissociated and sorted via fluorescent activated cell sorting (FACS). Subsequently, bulk RNA sequencing and specialised microRNA library-construction sequencing takes place. Bioinformatically, to process the sequencing data, a series of filtering and differential analysis steps are used to find the piRNAs and microRNAs that are believed to be relevant to the germline. The final step involves determining the functional, developmental and morphological importance of smallRNAs for the sea urchin germ line with the characterisation of gene expression via *in situ* hybridisation.

Results and conclusions

We have successfully isolated the sMic cells and the analysis of the sequencing data is undergoing. Previous research has established the importance of smallRNAs (e.g. PIWI-like) for the germ line of the sea urchin, found within gene networks that are crucial for the formation of gonads in the adult. Thus, compiling a library of these smallRNA enriched cells may shed light on the cell specification mechanisms and genetic drivers in these pluripotent and stem-cell-like cells.

Thematic Areas

Macroevolution

Insights into the evolution of the iconic notothenioid adaptive radiation of Antarctic fish based on 24 new genome assemblies

Iliana Bista^{1,2}, Antarctic Notothenioids fish genomes project Consortium², Eric Miska^{2,3}, Richard Durbin^{1,2}

¹ University of Cambridge, Genetics, Cambridge, United Kingdom

² Wellcome Sanger Institute, Tree of Life, Hinxton, United Kingdom

³ University of Cambridge, Gurdon Institute, Cambridge, United Kingdom

Aims

Antarctic notothenioid fishes, the predominant fish group of the sub-zero degree Southern Ocean, represent a dramatic example of vertebrate adaptive radiation with >100 species diversifying in the last 15MY.

Methods

We have generated 24 new whole genome assemblies representing five notothenioid families to help understand the radiation and genome evolution in extreme cold.

For the basal non-Antarctic notothenioid *Cottoperca gobio* (Bovichtidae) (fCotGob3, size 609Mb, scaffoldN50 14.74Mb), and the highly derived Antarctic icefish *Pseudochanichthys georgianus* (Channichthyidae) (fPseGeo1, size 1.03G, scaffoldN50 43Mb) we have completed reference assemblies assigned to chromosomes. Gene completeness for these and three other long-read assemblies (*Trematomus bernacchii*, *Harpagifer antarcticus*, *Gymnodraco acuticeps*) averages 95%, and gene annotation identified 21,662 (fCotGob3) - 23,222 (fPseGeo1) coding genes.

Results

Comparative analysis demonstrated a 65% expansion in genome size from the basal to derived species, with size ranging from 609Mb – 1.02Gb. The majority of this size expansion can be attributed to transposable element (TE) insertions (repeat content 28-46%), with multiple TE classes contributing to the expansion (DNA elements: CACTA, PiggyBac, hAT; retrotransposons: Copia, Gypsy, I, Pao). With these highly contiguous assemblies we have reconstructed the history of the expansion of the antifreeze gene family among notothenioids and the degeneration of globin genes in the white-blooded icefishes.

Main conclusion

This work provides a deep genomic characterization of this iconic group and a platform to investigate the mechanisms of fish cold adaptation and genome evolution.

Thematic Areas

Adaptation, Macroevolution

Ecological and social constraints combine to promote evolution of non-breeding strategies in clownfish

Branconi Rebecca^{*1}, *Barbasch Tina A.*¹, *Francis Robin K.*¹, *Srinivasan Maya*², *Jones Geoffrey P.*², *Buston Peter M.*¹

¹ Boston University, Department of Biology, 5 Cummington Mall 101, Boston, MA 02215, United States

² ARC Centre of Excellence for Coral Reef Studies, and College of Science & Engineering, James Cook University, Townsville, 4811, Queensland, Australia

Aim

Individuals that forgo their own reproduction in animal societies represent an evolutionary paradox because it is not immediately apparent how natural selection can preserve the genes that underlie non-breeding strategies. Cooperative breeding theory provides a solution to the paradox: non-breeders benefit by helping relatives and/or inheriting breeding positions; non-breeders do not disperse to breed elsewhere because of ecological constraints. However, the question of why non-breeders do not contest to breed within their group has rarely been addressed. Here, we use an experimental approach to investigate the ecological and social drivers for the evolution of cooperative breeding in clownfish (*Amphiprion percula*).

Methods

To test the hypothesis that non-breeding individuals do not disperse to breed elsewhere because of strong ecological constraints in the form of risk of mortality during dispersal, we experimentally tested the critical prediction that non-breeding individuals will disperse when the risk of moving between anemones is reduced. To test the hypothesis that non-breeding individuals do not contest for breeding positions because of strong social constraints in the form of evictions of non-cooperative individuals, we experimentally tested the critical prediction that non-breeding individuals will contest for breeding positions when the probability of winning a contest is increased.

Results

Our results show it is the combination of ecological and social constraints that promote the evolution of non-breeding strategies: non-breeders will disperse when ecological constraints (risk of mortality during dispersal) are experimentally weakened. In addition, we show non-breeders will contest when social constraints (risk of eviction during contest) are experimentally relaxed.

Main Conclusions

The findings highlight parallels between, and potential for unification of, cooperative breeding theory and economic bargaining theory: individuals will forgo their own reproduction and wait peacefully to inherit breeding positions (engage in cooperative options) when there are harsh ecological constraints (poor outside options) and harsh social constraints (poor inside options).

Thematic Areas

Adaptation, Populations and Species

Competitive Growth in a Social Fish

Branconi Rebecca¹, Reed Cymone, Desrochers Leah, Buston Peter

Boston University, Department of Biology, 5 Cummington Mall, Boston, MA 02215, United States

Aim

Many animal societies have dominance hierarchies in which social rank is correlated with size. In such societies, the growth and size of individuals can be a strategic response to their social environment: in fishes, individuals may decrease their growth rate to remain small and retain a subordinate position; in mammals, individuals may increase their growth rate to become large and attain a dominant position—a strategy called competitive growth. Here, we investigate whether the clown anemonefish, *Amphiprion percula*, exhibits competitive growth also.

Methods and Results

We show that juvenile clownfish paired with a size-matched reproductive rival increase their growth rate and size relative to solitary controls. Remarkably, paired individuals achieved this, despite being provided with the same amount of food as solitary controls. Subsequently, we use a suite of experiments manipulating availability of sensory cues to determine which cues must be available for individuals to engage in competitive growth. We show that mechanosensory (pressure and/or touch) cues or unobstructed interactions are necessary for competitive growth to occur.

Main Conclusions

Our results demonstrate that clownfish are able to increase their growth rate in response to social competition, and likely this process is mediated via mechanosensory cues. This study adds to the growing body of evidence that the growth of social vertebrates can be a fine-tuned plastic response to their social environment.

Thematic Areas

Adaptation, Populations and Species

Nitric Oxide and Retinoic Acid pathways crosstalk during amphioxus development

Caccavale F¹, Annona G¹, Subirana L², Escriva H², Bertrand S², D'Aniello S^{1,}*

¹ Department of Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn Napoli, Napoli, Italy.

² Sorbonne Université, CNRS, Biologie Intégrative des Organismes Marins (BIOM), Observatoire Océanologique, Banyuls-sur-Mer, France.

Aim

Chordate body axis patterning is finely regulated by complex signaling pathways. Retinoic Acid (RA) is a potent morphogen playing a pivotal role in vertebrate development.

Results

Here we demonstrate the functional cooperation between RA and Nitric Oxide (NO) occurring in amphioxus embryogenesis, the latter is widely known as a gaseous neurotransmitter and homeostasis molecule. We show that during amphioxus neurulation, NO modulates RA production through the transcriptional regulation of *Aldh1a.2* that irreversibly converts retinaldehyde into RA. On the other hand, RA regulates the transcription of *Nos* genes, probably through RA Response Elements (RARE) found in their regulatory regions.

Main Results

The reciprocal regulation of NO and RA pathways results to be essential for the normal pharyngeal development in amphioxus and we speculate that this regulatory crosstalk could be conserved in vertebrates.

Thematic Areas

Macroevolution

Global distribution of pelagic Tunicates

Luigi Caputi*, Roberta Piredda†, Salvatore D’Aniello*, Paolo Sordino*

*: Stazione Zoologica Anton Dohrn, Biology and Evolution of Marine Organisms Department

†: Stazione Zoologica Anton Dohrn Integrative Marine Ecology Department

Aim

Pelagic tunicates (Appendicularia, Thaliacea) play a key role in the ocean food web and carbon export towards lower compartments. Their global distribution is poorly known and we still possess an incomplete knowledge of how abiotic and biotic factors influence their diversity and abundance. Herein, we used metabarcoding data to build the first atlas of pelagic tunicates in oceans., We dedicated a specific focus on the response to local conditions and on the interaction with local planktonic communities.

Methods

We took advantage of the TARA Ocean V9 database to build a tunicates-specific V9 dataset. To do so, a new reference database was developed. OTUs delimitation was performed as a phylogenetic-based approach, which minimizes the uncertainties in the taxonomic assignation. To investigate the role of biotic and abiotic variables in triggering pelagic tunicates distribution, we used a machine learning approach (Boosted Regression Tree - BRT).

Results

Herein, the global distribution and abundance of 48 pelagic tunicate genera in the open ocean are presented. Pelagic tunicate abundance is associated with levels of Photosynthetically Active Radiation (PAR) and with seawater temperature. Moreover, biotic interactions with potential organic food and competitors contribute in determining the abundance of pelagic tunicates in the ocean. Tentatively, we have modelled Appendicularia and Thaliacea distribution in the framework of climate change. Preliminary results indicate that global warming may trigger an increase in pelagic tunicate abundance in the ocean.

Main conclusions

Tunicates are among the major component of macro-zooplankton, thus playing a fundamental role in the oceanic foodweb. We firstly created a global oceanic distribution map of pelagic tunicates, and determined the relative importance of various environmental drivers. Finally, we provide hints into how climate changing-related ocean warming may shape the future distribution of this important group of zooplankton.

Thematic Areas

Adaptation, Biodiversity, Zoology

Symbiont recognition and establishment in the nudibranch *Berghia stephanieae*

Jenny Melo Clavijo¹, Sabrina Bleidißel¹, Angelika Preisfeld¹, Gregor Christa¹

¹Bergische Universität Wuppertal, Fakultät für Mathematik und Naturwissenschaften, Zoologie und Biologiedidaktik, Wuppertal, Germany

Aim

The ability to associate with photosynthetic organisms and establish a photosymbiosis, is present throughout the animal kingdom. While in most photosymbiotic animals, the symbiont is commonly acquired directly from the environment, in nudibranchs the symbiont is sequestered from the food source, usually photosymbiotic cnidarians. Once the prey is ingested, the symbiont is selectively kept in epithelial cells of the nudibranch's digestive gland system. How the symbiont is recognized and not digested along with the photosymbiotic prey, and how the symbiont is maintained intracellularly are questions still unknown in the model Nudibranchia-Photosymbiont.

Methods

To approach these questions, we used the aeolid nudibranch *Berghia stephanieae* as a model organism. This photosymbiotic nudibranch feeds exclusively on sea anemones from the genus *Exaiptasia*, that harbor the dinoflagellate *Symbiodinium* as an intracellularly symbiont. We sequenced the transcriptome of *B. stephanieae* under three conditions: continuously fed, starved for seven days, and re-fed after seven days of starvation. We analyzed the gene expression focusing on the genes related to the innate immune system that have been previously linked to recognition and maintenance in photosymbiotic animals.

Results

We found potential recognition receptors that were upregulated when *B. stephanieae* fed after seven days of starvation. Among them are the scavenger receptor class B (SR-B), class E (SR-E), C-type lectins, toll-like receptors, proteins with a thrombospondin domain (TSRs), and complement C3. In contrast, we found down-regulated elements of the TGF β pathway, which is thought to be involved in the symbiont tolerance and maintenance. We also detected down-regulation in other components that inhibit phagocytosis.

Main conclusions

The symbiont recognition machinery in *B. stephanieae* might be similar to other photosymbiotic animals, however it seems that known pathways involved in symbiont maintenance and tolerance are not activated in *B. stephanieae*, suggesting a continuous symbiont digestion.

Thematic areas

Adaptation

Early developmental stages of native populations of *Ciona intestinalis* under increased temperature are affected by local habitat history

Clutton, E.A.^{1*}, Alurralde, G.^{2,3+}, Repolho, T.^{4*}

¹ Institute of Marine Sciences, University of Portsmouth, United Kingdom.

² Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Ecología Marina, Córdoba, Argentina.

³ Instituto de Diversidad y Ecología Animal (CONICET), Córdoba, Argentina.

⁴ MARE - Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Portugal

Aims

High levels of pollutants in anthropogenically-modified marine environments may exert high pressure on maximum physiological responses of sessile organisms acting as powerful agents of selection. Thus, an organism's acclimation or adaptive potential could play an important evolutionary role by enabling or conditioning species tolerance to stressful environmental conditions. Temperature modulates marine ectotherm physiology, influencing survival, abundance and species distribution. While native species could be susceptible to ocean warming, thermal tolerance might favour the spread of non-native species. Determining the success of invasive species in response to climate change is confounded by the cumulative, synergistic or antagonistic effects of environmental drivers, which vary at a geographical and temporal scale.

Methods

We investigated the developmental performance of early life stages of the ascidian *Ciona intestinalis* (derived from populations of anthropogenically-impacted and control sites) to an extreme weather event (i.e. marine heat wave). Fertilisation rate, embryo and larval development, settlement, metamorphosis success and juvenile heart beat rate were assessed as experimental endpoints.

Results

With the exception of fertilization and heart beat rates, temperature influenced all analysed endpoints. *C. intestinalis* derived from control sites were the most negatively affected by increased temperature conditions. Oppositely, *C. intestinalis* from anthropogenically impacted sites showed an overall positive response to thermal stress, with a higher proportion of larvae development, settlement and metamorphosis success being observed under increased temperature conditions. No differences were observed for heart beat rates between sampled populations and experimental temperature conditions. Moreover, interaction between temperature and populations was statistically significant for embryo and larvae development, and metamorphosis.

Main conclusion

We hypothesize that selection resulting from anthropogenic forcing could shape stress resilience of species in their native range and subsequently confer them advantageous traits underlying their invasive potential.

Thematic Areas

Adaptation, Populations and Species

Evolution of the TRH neuropeptide pathway and its function in the regulation of echinoderm larval growth

Maria Cocurullo*, Natalie Wood§, Paola Oliveri§, Maria Ina Arnone*

* Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Italy

§ Centre for Life's Origins and Evolution, University College London, United Kingdom; Research Department of Genetics, Evolution and Environment, University College London, United Kingdom

Aim

Neuropeptides are small intercellular signalling molecules generally produced by neurons and they regulate animal physiology and behaviour. For example, one of such neuropeptides is the Thyrotropin-Releasing-Hormone (TRH), an hypothalamic tripeptide, that controls metabolism, growth and reproduction acting through the Hypothalamic-Pituitary-Thyroid axis. Neuropeptides evolutionary origin can be tracked back to the common ancestor of bilaterian animals (ubilateria, >550 Mya) and the study of their evolution and function may shed light on the nervous system evolution and our understanding of its structure and function. Aim of our study is to take advantage of the phylogenetic and physiological characteristics of the sea urchin *Strongylocentrotus purpuratus* to investigate the TRH function in this deuterostomian invertebrate species in order to shed light on the evolution of the TRH pathway.

Methods

We characterized the role of a sea urchin TRH neuropeptide (QYPGamide), its precursor (SpTRH) and receptor (SpTRHR) genes in *Strongylocentrotus purpuratus* using a combination of chromogenic and fluorescent *in situ* hybridisation, whole-mouth immunostaining, morpholino antisense knock-down and CRISPR/Cas9 knock-out techniques.

Results

In sea urchins at early pluteus stage, SpTRH is produced by two cells bilaterally distributed at each the sides of the larval apical organ. These cells are also expressing the photoreceptor gene, Go-opsin (opsin 3.2). Our results show that knock-down and knock-out experiments of the SpTRH precursor and SpTRHR inhibit the post embryonic growth of the the skeleton supporting the larval arms, but has no consequences on the skeletal body rods.

Main conclusion

Our functional analysis of TRH in a non-chordate deuterostomes shows a conserved ancestral role of TRH neuropeptide on regulation of postembryonic growth, validating the hypothesis suggested in 2017 by Van Sinary et al. (Proc Natl Acad Sci U S A. 2017 114: E4065-E4074).

Thematic Areas

Macroevolution, Adaptation

Molecular Biomineralization of Octocoral Skeletons: Calcite vs. Aragonite

Conci N¹, Vargas S¹, Griesshaber E², Lehmann M³, Schmahl W², Wörheide G^{1,4,5}

¹ Department of Earth and Environmental Sciences, Palaeontology & Geobiology, Ludwig-Maximilians-Universität, Munich, Germany.

² Department of Earth and Environmental Sciences, Crystallography, Ludwig-Maximilians-Universität, Munich, Germany.

³ Biozentrum der LMU München, Department of Biology I-Botany, Grosshaderner Strasse 2-4, Planegg-Martinsried, Germany.

⁴ GeoBio-Center LMU, Ludwig-Maximilians-Universität, Munich, Germany

⁵ SNSB - Bayerische Staatssammlung für Paläontologie und Geologie, Munich, Germany

Aim

Biomineralization is the process by which organisms form minerals. Coral (Cnidaria: Anthozoa) biomineralization has been extensively studied in members of the order Scleractinia, primarily due to their role as framework-builders of coral reefs. However, from a biomineralogical perspective, scleractinians are quite uniform, as they all form aragonite exoskeletons. Contrarily, subclass Octocorallia (soft corals) produce diverse skeletons, including structures composed of different calcium carbonate (CaCO₃) polymorphs, i.e., calcite and aragonite. However, how corals can biologically control different polymorphs deposition remain elusive. This has important evolutionary implications, as changes in seawater chemistry through Earth's history, especially the Mg/Ca ratio, appear to favour the deposition of different polymorphs.

Methods

Here, we combined transcriptomics and proteomics to characterize the skeletal proteome of two calcite and one aragonite-forming octocoral and one scleractinian species.

Results

We found low proteome overlap between octocorals depositing different polymorphs, despite instances of shared proteins (e.g., galaxins and carbonic anhydrases). We additionally recreated proposed 'Calcite Sea' conditions (i.e., Mg/Ca of 2.5 and 1.5) in marine aquaria to culture the aragonitic octocoral *Heliopora coerulea* and scleractinian *M. digitata*. This revealed a species-specific response with *H. coerulea* maintaining the ability to produce aragonite, while up to 10% calcite was observed in *M. digitata* skeletons. RNA sequencing showed expression changes for calcium channels, pointing to possible responses of *H. coerulea* to lower Mg/Ca ratios.

Main conclusion

In conjunction, these data provide insights on how the diversity of coral skeletons evolved and may inform predictions about the response of these ecologically important organisms to future changes in ocean chemistry.

Thematic Areas

Adaptation, Macroevolution

Potential crypticity within two decapod (Crustacea) genera: *Galathea* (Fabricius, 1792) and *Eualus* (Thallwitz, 1891)

*Sheena Conforti*¹, *Federica Costantini*¹

¹ University of Bologna (Ravenna Campus) - Department of Biological, Geological, and Environmental Sciences (BiGeA)

Aim

Correct species identification and description are fundamental to understand health status of marine ecosystems. However, the use of a single identification tool for species distinction can lead to species misidentifications, having major consequences on ecological studies.

Methods

Here, we used an integrative taxonomic approach to identify benthic decapods belonging to the genera *Galathea* (Fabricius, 1793) and *Eualus* (Thallwitz, 1891) collected in the Mediterranean Sea. 23 *Galathea* and 22 *Eualus* individuals were morphologically analysed and sequenced at the mitochondrial COI gene to confirm their identity using BOLD Identification Engine.

Results

Morphological identification revealed the presence of two *Galathea* and three *Eualus* species, while species delimitation based on DNA barcoding of COI sequences revealed the presence of three *Galathea* and four *Eualus* species. Molecular analyses suggested the potential presence of two still undescribed species: one cryptic to *Galathea squamifera* and one cryptic to *Galathea intermedia*. Contrasting results obtained by morphological identification and BOLD Identification Engine impeded the recognition of *Eualus* specimens and suggested misidentifications among BOLD reference records of *Eualus cranchii*, *Eualus occultus* and *Eualus pusiolus*.

Main conclusion

These results demonstrated that morphological identification overlooks cryptic species and that misidentifications often occur, highlighting the importance of using an integrative approach to increase the current taxonomic knowledge of benthic invertebrates.

Thematic Areas

Biodiversity, Populations and Species

Integrated diversity assessment of zooplankton species in the Gulf of Naples

Rosa D'Angiolo, Marco Uttieri, Anna De Maio, Isabella Percopo, Carmen Minucci, Ferdinando Boero, Iole Di Capua

Aim

Zooplankton molecular analysis by DNA-barcoding allows for species identification with a proper molecular signature, and complements classic phenotypic taxonomy. This study underlines the effectiveness of DNA-barcoding to assess the biodiversity of the zooplankton community of the Gulf of Naples.

Methods

We analysed more than one hundred organisms, mostly Copepods, the main components of the Mediterranean zooplankton.

Results

After taxonomy identification, carried out by morphological analysis, DNA extraction of single organisms was performed and metazoan barcode was obtained by amplification of the mitochondrial gene, cytochrome c oxidase I (COI). We obtained the first COI *consensus* sequences from the Gulf of Naples for eight copepod species, three euphausiids, and one chaetognath.

Main conclusion

We identified individuals at different stages of development such as eggs and larvae of *E. krhonii*. The COI sequences will be deposited in GenBank and be used as reference sequences for other zooplankton works through DNA barcoding.

Thematic Areas

Biodiversity

Comparative analysis of gene networks in marine and terrestrial angiosperms

Emanuela Dattolo*, Luca Ambrosino*, Miriam Ruocco*, Gabriele Procaccini*, Maria Luisa Chiusano*§

¹ *Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

§Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (Na), Italy

Aim

Seagrasses are monocotyledonous angiosperms that live completely submerged in marine and estuarine environments. Phylogenetic analysis of members of the entire order indicates that the return into the sea occurred at least three times independently through parallel evolution from a common aquatic-freshwater ancestor of terrestrial origin during the Cretaceous period. The increasing availability of genomics and transcriptional resources for seagrasses enables the application of comparative genomics approaches to investigate ecological and physiological adaptations of these peculiar group of flowering plants.

Sequence comparisons among terrestrial and marine photosynthetic species provide the opportunity to explore gene and genome evolution in each of the independent seagrass lineages as well as the parallel evolutionary processes required for a fully marine lifestyle.

Methods

To achieve this goal, we constructed networks of orthologs and paralogs based on protein sequences comparison of eight species representatives of terrestrial and marine photosynthetic lineages (i.e. *Ostreococcus lucimarinus*, *Physcomitrella patens*, *Arabidopsis thaliana*, *Oryza sativa*, *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Zostera muelleri*), exploiting available genome and transcriptome data from public collections. To support the comparative analysis, we performed a functional annotation analysis by scanning the Swiss-Prot protein collection.

Results

We identified homologs relationships between each pair of analysed species, providing the first inventory of orthologs and paralogs gene networks of the four seagrass species and their relatives. The result showed that several gene families in each species exhibited characteristic features, suggesting their phylogenetic relationship and the potential divergence in their functions, while other gene families displayed a common pattern of differentiation among species.

Main conclusion

These efforts expanded molecular resources for understanding gene and genome evolution in seagrasses, highlighting peculiarities and conservation patterns among the different marine lineages and providing new data for future comparative functional analyses.

Thematic Areas

Adaptation, Macroevolution

Empirical evidence for concerted evolution in the 18S rDNA region of the planktonic diatom genus *Chaetoceros*

Daniele De Luca^{*†}, *Wiebe HCF Kooistra*^{*}, *Diana Sarno*[‡], *Elio Biffali*[‡], *Roberta Piredda*^{*}

^{*}Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

[†]Current address: Botanical Garden of Naples, Department of Biology, University of Naples Federico II, Via Foria 223, 80139 Naples, Italy

[‡]Department of Research Infrastructure for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

Aim

Concerted evolution describes the mode of evolution of certain repetitive sequences that evolve in concert within a genome. Classically, concerted evolution has been inferred indirectly from phylogenetic studies as “noise” in electropherograms and analysed through cloning and sequencing of limited subsamples of PCR-amplified products. We designed an experiment to reveal the occurrence of concerted evolution by means of metabarcoding data and discuss its potential impact on ecological and evolutionary studies.

Methods

We performed high-throughput sequencing (HTS) of the V4-18S rDNA region of monoclonal strains of six *Chaetoceros* species (marine planktonic diatoms) and analysed them together with a time-series environmental metabarcode dataset and *Chaetoceros* Sanger reference barcodes previously generated. Data were analysed by means of abundance plots, analysis of similarity and phylogenetic haplotype networks.

Results

The 18S rDNA region was under concerted evolution in the *Chaetoceros* species here analysed and that homogenisation was highly efficient at maintaining nearly identical 18S rDNA copies. Some intragenomic variation represented by the minor haplotypes was observed at the threshold of similarity of 99% with respect to a dominant haplotype. Minor haplotypes were not just technical artefacts because they were found in multiple independent environmental samples as well as in single strains obtained from the same locality. The distribution of haplotypes in both time-series and single strain data fitted Zipf’s law.

Main conclusion

Concerted evolution occurs in *Chaetoceros*. The dominant haplotype perfectly matches the Sanger reference sequence, validating the use of the metabarcoding technique for ecological studies. We highlight that the high number of sequences occurring at low abundances (minor haplotypes) could inflate diversity assessments, but they are intragenomic variation.

Thematic Areas

Macroevolution, Populations and Species

Molecular and morphological identification of *Pseudodiaptomus marinus* strains from Italian waters

Iole Di Capua, Rosa D'Angiolo, Elisa Camatti, Ylenia Carotenuto, Alenka Goruppi, Alessandra de Olazabal, Marco Pansera, Isabella Percopo, Valentina Tirelli, Giacomo Zagami, Marco Uttieri

Aim

Pseudodiaptomus marinus Sato, 1913 is a non-indigenous species (NIS) of the Mediterranean Sea, coming from Indo-Pacific region. This calanoid copepod was recorded in three Long Term Ecological Research sites (LTER). The first record was observed in the Gulf of Trieste (LTER_EU_IT_056) in 2007, and subsequently in Lake Faro (Messina) in 2008. It was further collected in the Gulf of Naples (LTER_EU_IT_13), and then it was observed also in the Venice Lagoon (LTER_EU_IT_016) in 2014.

Methods

In this study we performed an integrated morphological and molecular identification of *P. marinus* specimens from the four above mentioned sites.

Results

The morphological identification was carried out using the species-specific taxonomic keys available from the literature, while the molecular characterization was performed using the amplification of the mitochondrial gene cytochrome c oxidase I (COI).

Main conclusions

COI sequences of Italian specimens, compared with GenBank references, returned an identity between 97% and 100%. As added value, the COI sequences obtained in this study are the first ones for Mediterranean Sea *P. marinus*, representing a key benchmark for future studies including large-scale population genetic structure and connectivity.

Thematic Areas

Populations and Species

Stress granules in *Ciona robusta*: molecular evolution of TIA-1-related nucleolysin and tristetraprolin and gene expression studies under metal-induced stress conditions

Drago Laura, Peronato Anna, Franchi Nicola, Ballarin Lorian, Bakiu Rigers*, Santovito Gianfranco

Department of Biology, University of Padova, Padova, Italy

*Department of Aquaculture and Fisheries, Agricultural University of Tirana, Tirana, Albanian

Aim

Stress granules (SGs) are cytoplasmic foci, composed of non-translating messenger ribonucleoproteins, translational initiation factors and other additional proteins, able to modulate gene expression when cells are subjected to adverse environmental conditions. Very few works have been devoted to study the presence of the molecular components of SGs in invertebrates. In this work, we characterized the transcript sequences for two important mRNA-binding proteins of SGs, TIA-1-related nucleolysin (TIAR) and tristetraprolin (TTP), in the solitary ascidian *Ciona robusta*.

Methods

The identified sequences for *tiar* and *ttp* transcripts allowed us to start a study on the molecular evolution of these proteins in metazoans and carried out the first studies on their gene expression under stress conditions induced by metals (Cu, Zn, Cd and Fe).

Results

For both the proteins, the obtained results do not match the accepted phylogenesis of tunicates as sister group of vertebrates. Data on mRNA expression levels, provided by qRT-PCR, show a generalized decrease at the second day of metal-exposure for both *tiar* and *ttp*, suggesting that the induced acute stress promotes the inhibition of the transcription for the two studied proteins and then the disassembly of SGs to release useful mRNA to face the stress. ISH analyses demonstrate that TIAR and TTP antisense riboprobes recognize circulating granular amoebocytes in the hemolymph, visible in both blood lacunae and tunic.

Main conclusion

The results obtained in this work increase our knowledge on the molecular evolution of anti-stress proteins in metazoans and emphasize the importance of the transcription of *tiar* and *ttp*, which represents an efficient physiological response allowing organisms to survive under stress conditions in the environment.

Thematic areas

Adaptation

Perceived complexity and distinct boundaries: a revision of calcareous sponge *Leucosolenia complicata* in the White Sea

Irina Ekimova^{1,6}, *Alexander Ereskovsky*^{2,3,4}, *Dimitry Schepetov*^{1,4}, *Daria Tokina*³, *Alexandra Koinova*^{1,2}, *Andrey Lavrov*¹

¹Lomonosov Moscow State University, Leninskie Gory 1-12, 119234 Moscow, Russia;

²Saint-Petersburg State University, Saint-Petersburg, Russia

³Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille University, CNRS, IRD, Avignon University, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France

⁴N.K. Koltzov Institute of Developmental Biology RAS, Vavilov str. 26, 119334 Moscow, Russia

Aim

During last decades, the integrative taxonomy became a most popular approach to define and describe taxa on different taxonomic levels. However, it is often hard to implement it in taxa with sparse or extremely variable morphological characters. Sponges of the genus *Leucosolenia* (Calcarea, Calcaronea) encounter more than 40 species distributed worldwide. The systematics history of this group is complicated with different species conceptions proposed by various researches. The main goal of our study is to revise the diversity and taxonomy of the genus *Leucosolenia* from the White Sea using integrative approach. The additional aims of the study are identifying phylogenetically significant morphological characters and proposing optimal sets of molecular markers for further researches of calcareous sponges.

Methods

Molecular sampling included more than 250 individuals from the White Sea (Russia), Netherlands and Brittany (France). Molecular study included 18S, 28S and H3 markers and implemented Bayesian and Maximum likelihood phylogenetic approaches. Species delimitation and intraspecific divergence were investigated with ABGD and TCS-based analysis. Morphological analysis included studies of external morphology, general skeletal morphology, spicule morphology and cytology using light and electron microscopy.

Results

Integrative analysis showed that diversity of the genus *Leucosolenia* in the White Sea is represented by four species: *L. variabilis*, *L. corallorrhiza* and two species new to science. *Leucosolenia complicata* is restricted to NE Atlantic. Examination of morphological characters revealed a strong correlation between morphology of studied *Leucosolenia* species and their position on the phylogenetic tree. Species differ in general skeletal organization, spicule set and cellular composition.

Main conclusion

The integrative taxonomical approach, which included molecular studies with three markers and detailed morphological studies, made it possible to clarify the hidden diversity the genus *Leucosolenia* in White Sea.

The study was supported by RSF n°17-14-01089 (ultrastructural studies), RFBR nos°19-04-00563 and 19-04-00545.

Thematic Areas

Populations and Species, Biodiversity

Speciation drivers in high latitudes: systematics and phylogeography of the nudibranch family Coryphellidae (Gastropoda: Nudibranchia)

Irina Ekimova¹, Ángel Valdés², Manuel António E. Malaquias³, Cessa Rauch³, Anton Chichvarkhin⁴, Anna Mikhlin¹, Tatiana Antokhina⁵, Dmitry Schepetov^{1,6}

¹ Lomonosov Moscow State University, Moscow, Russia

² Department of Biological Sciences, California State Polytechnic University, CA, USA

³ University Museum of Bergen, University of Bergen, Bergen, Norway

⁴ National Scientific Center of Marine Biology, Far Eastern Branch RAS, Vladivostok, Russia

⁵ A.N. Severtsov Institute of Ecology and Evolution, Moscow, Russia

⁶ N.K. Koltzov Institute of Developmental Biology RAS, Moscow, Russia

Aim

The nudibranch group Flabellinidae *s.l.* (Gastropoda: Nudibranchia) represents a highly diverse and widely distributed group of marine gastropods. Several independent flabellinid lineages occur in boreal regions with only a few representatives in tropical areas. In addition, most species show overlapping ranges and some of them occur sympatrically in very restricted areas. The main goal of this study was to conduct an updated revision of the family Coryphellidae (an independent lineage within Flabellinidae *s.l.*) using integrative approach.

Methods

The molecular study included five markers (COI, 16S, H3, 28S and 18S) and implemented Bayesian and Maximum likelihood phylogenetic approaches. Species delimitation and intraspecific divergence were investigated with ABGD, PTP and GMYC methods, and TCS-based haplotype analysis. Morphological analysis included standard anatomical techniques and scanning electron microscopy. Ancestral area reconstruction and divergence time estimation were implemented to uncover evolutionary history of this group.

Results

Based on integrative analysis, we propose a new classification system for the family Coryphellidae and suggest criteria for further revision of flabellinid taxa. Ancestral area reconstruction (AAR) provides evidence for a tropical Pacific origin of the genus. We conclude that the evolution of *Coryphella* has been shaped by different processes: initial migration out of the tropics, radiation in the North Pacific influenced by Miocene climate change, and subsequent allopatric speciation resulting from successive closings of the Bering strait and cooling of the Arctic Ocean during the Pliocene-Pleistocene.

Main conclusion

This study illustrates a complex evolutionary history, in which multiple, independent processes during different periods of time, produce an unusual biogeographic pattern. This model should be tested in other groups of marine invertebrates with similar diversity patterns to better understand the mechanism of generation of biological diversity in non-tropical regions.

This study was supported by Russian Science Foundation grant #20-74-10012.

Thematic Areas

Populations and Species, Biodiversity

Gene body and environmentally inducible DNA methylation in seagrasses: inter- and intraspecific differences and associations with transcriptome plasticity under warming conditions

Laura Entrambasaguas^{}, Miriam Ruocco^{*}, Koen J.F. Verhoeven[†], Gabriele Procaccini^{*}, Lazaro Marín-Guirao^{*,‡}*

^{*} Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.

[†]Terrestrial Ecology Department, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, the Netherlands.

[‡]Seagrass Ecology Group, Oceanographic Center of Murcia, Spanish Institute of Oceanography, C/ Varadero, 30740 San Pedro del Pinatar, Spain.

Aim

Cytosine methylation is a widespread epigenetic modification found in eukaryotes. Methylated CG is the most abundant in plant genomes and generally occurs in repetitive/noncoding regions. Coding regions are also methylated and several studies have found that methylation of gene bodies (gbM) varies according to gene function, where genes with housekeeping functions tend to be more heavily methylated than those with inducible functions. The aim of the present study is to provide a comprehensive evaluation of DNA methylation and gene expression patterns in two marine plants with contrasting life history strategies for understanding the role of epigenetic mechanisms in the adaptation to local environments and in the response to heat stress.

Methods

We assessed gbM thorough the *in silico* transcriptome analysis of CpG_{O/E} ratios in the two Mediterranean seagrass species *Posidonia oceanica* and *Cymodocea nodosa* collected along a latitudinal gradient (cold and warm environment) and undergoing a heat stress treatment.

Results

We demonstrate that the bulk of *Posidonia oceanica* and *Cymodocea nodosa* genes possess high levels of intragenic methylation. We also observed a correlation between low gbM and gene expression flexibility between experimental temperature regimes and between plants from different populations.

Main conclusion

The empirical determination of global DNA methylation showed patterns of intra and inter-specific divergence that indicate a relationship between methylation level and the plants' latitude of origin and life history. We reinforced the hypothesis that low DNA methylation facilitates flexible expression and plasticity under changing conditions, with a lower methylation level in genes with inducible functions.

Thematic Areas

Adaptation, Populations and Species

Becoming a limpet: an ‘intermittent limpetization’ process driven by host features in the kleptoparasitic gastropod family Capulidae

*Giulia Fassio**, *Philippe Bouchet*[§], *Pierre Lozouet*^{§§}, *Maria Vittoria Modica*[¶], *Valeria Russini**,
Stefano Schiaparelli^{¶¶}, *Marco Oliverio*^{*}

* Department of Biology and Biotechnologies “Charles Darwin”, Sapienza University of Rome, Rome, Italy

§ Institut de Systématique, Évolution, Biodiversité, ISYEB, UMR7205 (CNRS, EPHE, MNHN, UPMC), Muséum National d’Histoire Naturelle, Sorbonne Universités, Paris, France

§§ Muséum National d’Histoire Naturelle, Direction des Collections, Paris, France

¶ Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy

¶¶ Department of Earth, Environmental and Life Science (DISTAV) – and – Italian National Antarctic Museum (MNA, Section of Genoa), University of Genoa, Genoa, Italy

Aim

A coiled shell is the most evident feature of the Bauplan of a gastropod mollusc. However, at least 54 families independently evolved an apparently simplified shell morphology: the limpet. Species with this largely uncoiled, depressed shell morphology occur in almost every aquatic habitat and are associated to several different lifestyles and diets. The marine gastropod family Capulidae includes 18 recognised genera, the large majority of which are coiled, but with a number of limpet-like species. Capulid shell plasticity is also associated to a broad range of feeding ecologies, from obligate suspension feeders to kleptoparasites. Given the plasticity of shell shapes and the array of feeding strategies of capulids, this family is an ideal model to investigate (a) the evolution of limpet-like shells, (b) the evolution of kleptoparasitic feeding strategies and (c) the relationship between the two traits.

Methods

To investigate the evolution of the limpet-like shell in the family Capulidae we performed an ancestral state reconstruction analysis on a time-calibrated phylogenetic tree (COI, 16S, and ITS2) including 16 species representing a good deal of its morphological diversity.

Results

Our results identified at least three capulid lineages that independently evolved limpet-like shells, suggesting that a recurrent limpetization process characterizes this family. At least one of the limpet-like genera was undescribed and deserves the introduction of a new genus-level taxon.

Main conclusions

We suggest that capulids evolved from a lineage of coiled suspension feeders and that the shift to kleptoparasitism in the family ancestor may have represented a strategy to save energy through the exploitation of the water current produced by the host. Probably the major drivers of shell evolution in capulids are related to their ecology, most of them being kleptoparasites, include the shape and the kind of host substrate, and lead to the repeated acquisition of a limpet-like shape.

Thematic areas

Macroevolutions, Adaptation

The molecular assembly of vision

Roberto Feuda¹

¹Department of Genetics and Genome Biology, University of Leicester, UK

Aim

The vision is a fundamental aspect of our daily life, and its evolution has puzzled scientists since Darwin. At the molecular level, it requires a sophisticated molecular machinery that is encoded by different genes. This process starts with photons of light activating a photosensitive photopigment, typically an opsin gene. This event results in the activation of a phototransduction cascade that transforms the light information into an electric signal. Finally, to function in the visual process, all these genes need to be expressed in a specialized photoreceptor cell.

Methods

We integrated phylogenetics methods and single-cell genomics to understand when the molecular components of vision evolved.

Results and conclusion

The result indicates that vision in metazoan evolved gradually, and that several molecular components largely predate the origin of eyes.

Thematic areas

Macroevolution

The Internal Transcribed Spacer 2 (ITS2) as a new barcoding marker in nudibranchs (Gastropoda: Heterobranchia): an approach using sequence and secondary structure information

Matteo Garzia^{1†}, Paolo Mariottini¹, Daniele Salvi², Giulia Furfaro^{3†}*

¹ Department of Health, Life and Environmental Sciences, University of L'Aquila, I-67100 Coppito, L'Aquila, Italy

² Department of Science, University of Roma Tre, I-00146 Rome, Italy

³ Department of Biological and Environmental Sciences and Technologies - DiSTeBA, University of Salento, I-73100 Lecce, Italy.

Aim

In this study we assess the performance of the nuclear ITS2 rRNA marker for species identification and species delimitation in eolid nudibranchs. We also characterized the ITS2 rRNA secondary structure of each taxa that can provide additional taxonomic resolution.

Methods

ITS2 rRNA multiple sequence alignments were performed simultaneously considering the secondary structure of each sequence. We compared ITS2 pairwise genetic distance (*p*-distance and distance corrected by the Kimura two parameters model) between 14 species representing 10 genera within six eolid families with distances calculated with the mitochondrial markers COI and 16S rRNA. We infer the ITS2 secondary structures by contrasting several candidate low free energy folding models and we assess the presence of CBCs (Compensatory Base Changes) and semiCBCs across the ITS2 dataset.

Results

Based on the ITS2 pairwise distance it is possible to discriminate species within the same genera. The common derived ITS2 rRNA secondary structure of the eolid nudibranchs is organized in five domains, D1-D5, and in the D5 we identified the molluscan *consensus* Apical STEM. We also identified CBCs and semiCBCs that are diagnostic at both the species and family level.

Main conclusions

In this work, we propose the nuclear ITS2 rRNA marker as a new barcoder for eolid nudibranchs. We characterized for the first time the ITS2 rRNA secondary structure in nudibranchs and we pinpointed the conserved regions. Our results would desirably stimulate the increase of DNA barcoding libraries available for nudibranchs that currently only include mitochondrial DNA sequences.

Thematic Areas

Macroevolution, Biodiversity.

Growing evidence of massive gene presence-absence variation in bivalve mollusks: does it provide an adaptive value?

*Marco Gerdol**, *Samuele Greco**, *Alberto Pallavicini**

*Department of Life Sciences, University of Trieste, Italy

Aim

The increasing accessibility of next generation sequencing offers new opportunities for the genomic study of non-model organisms, allowing to extend such investigations to multiple individuals and to explore phenomena which have so far remained obscure. The aim of this work was to investigate the impact of the unusual architecture of the Mediterranean mussel (*Mytilus galloprovincialis*) genome on the high invasiveness, resilience and adaptation potential of this species.

Methods

We applied a whole genome resequencing approach to identify genes subject to presence-absence variation (PAV) in different individuals belonging to two independent populations. Resequencing data was mapped to the mussel reference genome assembly, allowing to detect hemizygous genomic regions and associated protein-coding genes subject to PAV. These *dispensable* genes were subjected to functional enrichment analyses, based on Gene Ontology and Pfam domain annotations, to highlight the terms most frequently associated with the accessory fraction of the genome.

Results

The genome of the Mediterranean mussel provides an unprecedented example of widespread gene PAV in Metazoa. The high levels of intraspecific genetic variation can be explained by massive structural variants that include very large hemizygous regions containing protein-coding genes subject to PAV. Overall, the mussel genome comprises 45,000 *core* genes found in all individuals and 20,000 *dispensable* genes, which are enriched in functions associated with immune response and survival.

Main conclusion

Preliminary analyses carried out on recently published, complete genomes from other species indicate that gene PAV is widespread in mollusks, and particularly prominent in bivalves. The functional enrichment of *dispensable* genes suggest that these represent useful tools for genomic evolution, allowing the maintenance of additional genetic diversity within the population and providing enhanced chance of survival in diverse environments.

Thematic Areas

Adaptation, Population and Species

The Pandora's Box: morphological and genetic diversity within the genus *Amphiglena* Claparède, 1864 (Sabellidae, Annelida) in the Mediterranean Sea

Adriana Giangrande ¹⁻², Matteo Putignano¹, Margherita Licciano ¹, Maria Cristina Gambi ²

1 DiSTEBa, University of Salento, Monteroni di Lecce, Lecce (Italy)

2 Stazione Zoologica Anton Dohrn, Dept Marine Integrative Ecology, Ischia Marine Center, Ischia (Napoli, Italy)

Aim

The very common and small sabellid polychaete *Amphiglena mediterranea* (Leydig, 1854) is known to be the only species of this genus reported in the whole Mediterranean Sea. However, preliminary genetic analyses within the Gulf of Naples, highlighted the occurrence of at least 5 putative sibling species.

Methods

Here we report the description of 9 new taxa within the genus *Amphiglena*, whose diversity in the Mediterranean basin has been widely underestimated. Examined material derived from both from new collections along the Italian coast, including four CO₂ vents/hydrothermal systems, and from a re-examination of older material previously attributed to *A. mediterranea*.

Results

The morphological analyses revealed the presence of different taxa also consistently with a previous molecular analyses conducted on material from the Gulf of Naples. This led to an increase in the number of species in the genus and to enhance the existence in the Mediterranean of a high diversity within the genus, showing also species found in quite unique environmental conditions, such as hydrothermal vent systems, where 5 of the 9 species have been described. Some taxa, however, remained for the moment undescribed due to the poor preservation of the old material, and the lack of the type material for this taxon.

Main conclusion

A larger revision of all the previous Mediterranean material previously attributed to *A. mediterranea* from both morphological and molecular point of views is needed.

Thematic Areas

Biodiversity, Populations and Species

Early stage of development of red coral (*Corallium rubrum*) skeleton: the key role of sclerites during the first years of life

Bruna Giordano^{*‡}, *Lorenzo Bramanti*[‡], *Johnathan Perrin*[†], *Daniel Vielzeuf*[§]

*CNRS-Sorbonne Université, Laboratoire d'Ecogéochimie des Environnements Benthiques, LECOB, Observatoire Océanologique de Banyuls sur Mer, 1 avenue Pierre Fabre, 66650 Banyuls sur Mer, France

‡Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro, 5, 00185, Rome, Italy

§ Aix Marseille Université, CNRS, Centre Interdisciplinaire de Nanoscience de Marseille, UMR 7325, Marseille, France

†Synchrotron SOLEIL, L'Orme des Merisiers, BP48, Saint Aubin, 91192 Gif-sur Yvette, France

Aim

In sessile benthic species, survival of early stages is critical for the resilience of the populations. To increase survival, species have developed different defense strategies. One of the most common is the production of hard structures like skeletons or spicules, as a protection from abrasion or predation. *Corallium rubrum* is an octocoral species endemic to the Mediterranean Sea which produces two types of biomineral CaCO_3 structures: the sclerites and the inner skeleton, composed by a medullar zone (MZ) surrounded by an annular domain (AZ). Skeletogenesis processes are well known for adult colonies but still poorly understood for the early life stages.

Methods

To understand the dynamics of the skeleton formation during the first 4-years of life, colonies of different ages (17-day-, 1-, 2-, and 4-years- old) have been observed with stereomicroscope and scanning electron microscope and analyzed by synchrotron tomography and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS).

Results

During the first year, colonies did not produce an axial skeleton, but a proto-skeleton made up of sclerites not embedded in the calcitic matrix. During the second year of life, a hard skeleton formed by coalescence of sclerites and calcitic cement is produced, which correspond to the MZ observed in adult colonies. LA-ICPMS analyses showed that the chemical signature of the skeleton of 4-years-old colonies is similar to the MZ adult colonies'one, suggesting that, at that age, annular CaCO_3 deposition still not occur.

Main conclusion

Our findings emphasize the importance of sclerites during the early developmental stages when they could offer mechanical protection from abrasion and predation forming a deformable, fast-growing armor that allows a safe development of the first polyps. Furthermore, sclerites could serve as nuclei for triggering the cementation and foster the formation of the hard axial skeleton.

Thematic Areas

Populations and Species, Biodiversity

Making of a pathogens: how marine microbial genomics can help understand how pathogens are made

Donato Giovannelli, Angelina Cordone

University of Naples Federico II, Naples, Italy

Aim

In the ocean, microbes influence biogeochemistry, climate and overall planet functioning. They outnumber any other living organism by far, with recent estimates of microbial biomass on Earth between 9 and 31×10^{29} cells, and control all major biogeochemical cycling of elements. Over time, they have influenced the overall redox state of the surface of our planet and permanently bioengineered the environment. Besides controlling biogeochemical cycles, microbes also affect human and animal health. Microorganisms that comprise both beneficial strains and potential pathogens colonize the exposed areas of our bodies and our gastrointestinal tract. Understanding how microbes have adapted to colonize every available environmental niche, including the outside and inside of other organisms, may hold the clue to a more efficient fight against microbial diseases. The reconstruction of the evolutionary history of key biochemical functions is critical to understand this process. Given that animals and mammals are comparatively new-comers compared to ocean ecosystems, especially geothermal influenced ones, it is plausible to hypothesize that that beneficial and pathogenic microorganisms that today colonize the inside and outside of higher organisms, including ourselves, have evolved recently. We will discuss how the comparative genomic of marine microbes can help understand the evolutionary journey of pathogens, and present a case study from the ubiquitous marine class Epsilonproteobacteria. Understanding which portion of the pathogens' metabolic machinery has been conserved and adapted to new conditions and what portion of their genomes is an acquired trait is fundamental in delineating the evolutionary journey from environmental strains to human pathogens.

Thematic Areas

Adaptation, Macroevolution

Intelligent networks: new perspectives in the application of Internet of Things for marine investigations.

*F Glaviano *†, T Viel *, M Mutalipassi *, M Costantini *, M Buia ‡, V Zupo **

*Marine Biotechnology Department, Stazione Zoologica Anton Dohrn, Naples, Italy; †Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Naples, Italy;

‡Integrative Marine Ecology (EMI) Department, Stazione Zoologica Anton Dohrn, Naples, Italy.

Aim

The Internet of Things (IoT) revolution opens totally new approaches to various scientific field due to its interconnected network made of physical objects able to communicate and cooperate with each other to develop the next generation of "intelligent" devices.

Biodiversity studies need continuous monitoring of marine environments and intelligent devices to reduce management efforts. The main objective of this study is to advance our understanding of ecological processes and trends and achieve a better grasp of a variety of systems, both natural and reconstructed, needing intelligent management.

Methods

This research intends to finalize the creation of a network of intelligent nodes integrated and organized for the monitoring, control and automation of processes at different levels of complexity. To this end, intelligent devices driven by IoT modules are going to be developed such as specifically designed tanks and photobioreactors as well as a network of probes designed to monitor the environmental parameters related to phanerogam meadows regression and deriving biodiversity loss.

Results

Unlike pre-existing automated systems, IoT implementation that we are testing can generate "interactive operators" capable of reading environmental signals, carry out assessments and respond with programmed actions. The potential of these intelligent systems will permit a much more precise, advanced and simple management to be integrated into scientific research.

Main conclusion

The data obtained through these IoT systems can provide fundamental information for biodiversity conservation planning and can help to better understand and face the changes that we are likely to face in the next future.

Thematic Areas

Biodiversity, Adaptation.

Climate change and the case of the Hydrozoa taxon in the Mediterranean Sea: biodiversity vs disappearance of species

Cinzia Gravili

Laboratory of Zoology and Marine Biology, Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce, Italy

Aim

The pressure of anthropogenic activities on marine ecosystem has increased in recent decades. Biodiversity assessment is often restricted to groups studied in greater detail, usually popular and charismatic species. The majority of biodiversity consists, on the other hand, in little known and inconspicuous species. The Hydrozoa taxon includes organisms that are particularly sensitive to climate change. The aim of this work is to review the knowledge about the diversity of the Mediterranean non-Siphonophora Hydrozoan (NSH) fauna and examine whether species might have become extinct.

Methods

The list of NSH species of the Mediterranean Sea is based on taxonomic revisions and recent papers. To determinate historical series and distribution, 785 faunistic studies published between 1850 and 2020 have been consulted and a database with 8,385 records was organized so as to provide the information.

Results

The list of NSH species of the Mediterranean Sea has about 400 species, including 69 non-indigenous species. Global warming is favoring the tropical contingent, while the boreal one is in a stressful condition. The change in biodiversity is often decoupled from the richness of species: the rate of introduction of alien species often exceeds that of the extinction of native species in the same habitat, leading to erroneous interpretations of local biodiversity. In fact, while some populations of hydroids are in decline or disappear (more than 10% of the Mediterranean NSH have not been reported in the last 40 years), other ones invade new areas and habitats.

Main conclusion

The species lists are dynamic and require continuous updating that considers both the subtraction of the species with non-contemporary reports and addition of the introduced species. In conclusion, the role of the naturalist in today's society should be re-evaluated by recovering traditional scientific methods such as long-term observations that provide valuable information on changes in ecosystems.

Thematic Areas

Biodiversity, Populations and Species

Transcriptomic adaptations of Cryonotothenioidea to the antarctic environment and response to heat stress

Samuele Greco[†], Anastasia S. Gaetano[†], Federico Ansaloni^{†§}, Gianfranco Santovito[‡], Andrea Miccoli^{*},
Giuseppe Scapigliati^{*}, Alberto Pallavicini[†], Piero G. Giulianini[†], Marco Gerdol[†]

[†] Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Trieste, Italy

[‡] Dipartimento di Biologia, Università degli Studi di Padova, Padova, Italy

^{*} Dipartimento di Scienze Ambientali, Università della Tuscia, Viterbo, Italy

[§] International School for Advanced Studies, Trieste, Italy

Aim

Antarctic fishes adopted a wide set of adaptations that allow them to thrive in the extreme antarctic environment. For example due to the high solubility of gasses in cold waters some *Channichthyidae* such as *Chionodraco hamatus* have completely lost hemoglobin and gasses are simply dissolved in their colorless blood.

Although other adaptations, such as an increased activity of carbonic anhydrase activity in gills, have been previously described from a biochemical point of view, these have not been investigated from a genomic perspective.

Here we explore cold adaptations of Cryonotothenioidea at a transcriptomic level in a multi-species comparison. Moreover we investigate the transcriptomic response of *Trematomus bernacchii* to a slight heat stress.

Methods

Whole transcriptome sequencing data from 14 fish species were collected from NCBI or generated *de novo*. A bioinformatic approach was used to identify peculiar transcriptomic features of antarctic species compared with species living in temperate environments, and to assess the response of *Trematomus bernacchii* to a slight heat stress in 3 different tissues (brain, gill, muscle).

Results

We identified 130 upregulated genes in the gills of Cryonotothenioidea, including two carbonic anhydrases displaying high gill-specificity. Moreover molecular signatures of cobalamin deficiency, which could be linked with the high parasite loads found in these species, were observed.

The heat stress experiment revealed the brain as the most sensible tissue after 20 days of exposure, with most changes affecting protein synthesis, vesicular transport, inflammation and cytoskeleton assembly. Comparatively, the gills and muscle tissues displayed an earlier and weaker transcriptomic response. Interestingly, several hsp were regulated in response to stress both from heat and stabling.

Main conclusion

This study allowed us to identify peculiar transcriptomic markers of cold adaptation in Cryonotothenioidea and to understand how the future sea water warming may affect the response of these organisms in the short term.

Thematic areas

Adaptation

Did genomics forget about Brachyura? A draft genome for the crab *Chiromantes haematocheir*

Alessio Iannucci¹, Giovanni Bacci¹, Andrea Benazzo², Duccio Cavalieri¹, Claudio Ciofi¹, Alessio Mengoni¹, Niccolò Meriggi¹, Stefano Cannicci^{1, 3}, Sara Fratini¹

¹ Department of Biology, University of Florence, via Madonna del Piano 6, 50019 Sesto Fiorentino, Italy.

² Department of Life Sciences and Biotechnology, University of Ferrara, 44121 Ferrara, Italy

³ The Swire Institute of Marine Science and Division of Ecology and Biodiversity, The University of Hong Kong, Pokfulam road, Hong Kong SAR.

Aim

The availability of high-quality reference genomes has led to great advancements in comparative and functional genomics and has uncovered several aspects of the evolution of physiological adaptations. Despite many reference genomes are available both for vertebrate and invertebrate species, some taxa remain deeply underrepresented. Among these taxa there is the decapod infra-order Brachyura, including all the extant true crabs, for which only two genomes have been assembled so far.

The deficiency of genomic data on Brachyura can certainly not be attributed to a lack of interest in this taxa, since its ecological and economic value is unquestionable. On the contrary, this depends on some critical technical difficulties in the assembly production due to crustacean genome organization. Here we present the preliminary steps we performed to assemble the genome of the tropical crab *Chiromantes haematocheir*. This species has a terrestrial life style, and it was specifically chosen to investigate the biological and physiological processes involved in the sea-land transition of brachyuran crabs.

Methods

Fundamental prerequisite to perform a correct assembly of sequencing data is to get information on the genome size and chromosome organization of the target species. For this, we measured the genome size of *C. haematocheir* using flow cytometry, and performed cytogenetic analyses to determine its chromosome number. Then we performed initial sequencing of *C. haematocheir* genome using PacBio facilities.

Results and Conclusions

Despite some technical difficulties, we managed to obtain a draft genome for *C. haematocheir*. Our research goal is to use the genome assembly to investigate the evolutionary processes involved in the sea-land transition of this species. Thus we will investigate possible genes, or groups of genes, selected under the environmental pressures due to terrestrialization, shedding light on the genomic adaptation that led marine crabs to adapt to a terrestrial ecosystem.

Thematic Areas

Macroevolution, Adaptation

Diversity of the Pterasteridae (Asteroidea) in the Southern Ocean: a molecular and morphological approach

Quentin Jossart^{1*}, Marc Kochzius¹, Bruno Danis², Thomas Saucedo³, Camille V.E. Moreau^{2,3}

Aim

An integrative approach is crucial in discrimination of species, especially for taxa that are difficult to identify based on morphological characters.

Methods

In this study, we combine genetics and morphology to assess the diversity of Pterasteridae, a sea star family diversified in deep-sea and polar environments. Because of their derived anatomy and the frequent loss of characters during preservation, Pterasteridae are a suitable case for an integrative study.

Results

The molecular identification of 191 specimens (mostly from the Southern Ocean) suggests 26–33 species in three genera (*Diplopteraster*, *Hymenaster* and *Pteraster*), which match the morphological identification in 54–62% of cases. The mismatches are either different molecular units that are morphologically indistinguishable (e.g. *Pteraster stellifer* units 2 and 4) or, conversely, nominal species that are genetically identical (e.g. *Hymenaster coccinatus/densus/praecoquis*). Several species are shared between the Northern and Southern Hemispheres (e.g. *Pteraster jordani/affinis*).

Main conclusion

In conclusion, the taxonomic status of some groups is confirmed, but for others we find the need to re-evaluate the taxonomy at both genus and species levels. This work significantly increases the DNA barcode library of the Southern Ocean species and merges taxonomic information into an identification key that could become a baseline for future studies (pterasteridae-so.identificationkey.org).

Thematic Areas

Biodiversity

Strong macrobenthic community differentiation among sub-Arctic deep fjords on small spatial scales

Valentin Kokarev, Henning Reiss

Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway

Aim

Fjords play an important role in carbon cycling and sequestration, but the burial of organic matter in sediments strongly depends on the composition of macrobenthic communities. We studied three deep sub-Arctic fjords located in northern Norway to assess the community differentiation of neighbouring fjords and the underlying environmental drivers. The fjords have relatively deep depositional basins (370-570 m), but they differ in sill depth and water exchange.

Methods

Overall, 36 grab stations were performed in three fjords to study the macrobenthic species composition. In addition, we performed CTD measurements and analyses of organic matter and grain size in the surface sediments.

Results

We found that each basin was inhabited by a distinct benthic community, with pronounced differences in total abundance, biomass and species diversity. Only in one fjord (Skjerstadvfjord) with a shallow sill, a slight mouth-to-head pattern of the community was found. The strong differentiation of macrobenthos among different fjords seems not to be directly associated with environmental drivers such as organic matter input, water masses or depth.

Main conclusion

Strong differentiation of macrobenthic communities suggests low environmental filtering of fauna and importance of stochastic and biological processes during community assembly.

Thematic area

Biodiversity

The DNA-Methylation machinery of the mollusc *M. galloprovincialis* and its essential role in development

La Vecchia Claudia¹, Gerdol Marco², Strazzullo Maria¹, De Luca Pasquale³, Gorbi Stefania⁴, Pallavicini Alberto¹⁻², Regoli Francesco⁴ and D'Aniello Enrico¹.

¹Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, 80121 Naples, Italy;

²Department of Life Sciences, University of Trieste, 34127 Trieste, Italy; ³Research Infrastructures for Marine Biological Resources Department, Stazione Zoologica, Anton Dohrn, Naples, Italy; ⁴Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy.

Aim

DNA methylation is an important epigenetic mechanism influencing gene expression in both vertebrates and invertebrates. The pattern of DNA methylation changes during development and embryogenesis. As a consequence, differentiated cells develop a stable and unique DNA methylation pattern that regulates tissue-specific gene transcription: a critical mechanism for development. DNA methylation however remains poorly investigated in molluscs.

Methods

To shed light on this process, we screened the genome of the mollusc *Mytilus galloprovincialis*, we first employed database search, detecting three major groups of DNMT enzymes, three MBD members and one TET protein, which were subject to phylogenetic studies and protein domain characterization. We then investigated the expression of these genes both during development and in different adult tissues together with the dynamics of global DNA methylation levels during embryogenesis and how a specific DNMT1 inhibitor, 5-aza-cytidine, influence the embryonic development.

Results

Our results provide a first insight on the importance of DNA methylation in *Mytilus* embryos and how impairment of this process give rise to aberrant and not fully developed embryos.

Main conclusion

Since epigenetic mechanisms are important in promoting phenotypic plasticity, detailed studies will be necessary to help to improve the efficiency of *M. galloprovincialis* husbandry, one of the most important aquaculture resources worldwide, and more generally of other Lophotrochozoans.

Thematic Area

Adaptation

***Ciona robusta* reveals specific gut immune adaptations to a microbial world**

*Assunta Liberti**, *Paolo Sordino** and *Larry J. Dishaw†*

* Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy

† University of South Florida, Morsani College of Medicine, Department of Pediatrics, Children's Research Institute, Saint Petersburg, FL, USA

Aim

The essential role of microorganisms in shaping animal physiology remains a topic of intense scrutiny. Specifically, the gut immune system is at the forefront of the crosstalk between host and microbes, where colonization by commensal microbes is tolerated while pathogens are resisted. This dialogue is evolving and is shaped by a continuum of microbial species. Establishing diverse model systems is essential for understanding the fundamental rules legislating these ecological interactions. Thus, *Ciona robusta*, an invertebrate chordate that interfaces with the environment using only innate immune adaptations, is a prime model system.

Methods

Diverse innate effectors have been described in *Ciona*; most are functional in the gut environment, a primary interface with the environment. I will focus on a family of secreted immunoglobulin-like effector molecules, the Variable Ig-containing Chitin-Binding Proteins, (VCBPs), which have been studied both using *in situ* hybridization and immunohistochemistry techniques, and functional assays; binding to diverse bacteria and fungi has been demonstrated using microbiological techniques.

Results and Main conclusion

The VCBPs are characterized by two Ig domains of the variable type (IgV), and a C-terminal chitin-binding domain (CBD); they are mostly expressed in blood and epithelial cells of the digestive tract. Among four VCBPs, VCBP-C has been most functionally characterized. It is shown to bind bacteria via the IgV domains and is opsonic; fungi can be bound via the CBD domain. This feature, to our knowledge, makes VCBP-C the first secreted Ig-containing immune molecule with the capacity to directly influence transkingdom interactions. Similar to mammalian IgA, VCBP-C is able to influence bacterial biofilm formation and likely modulates settlement in the gut. Based on these characteristics, *Ciona robusta* represents a model system that may help to define conserved and/or unique innate immune adaptations that shape the gut ecosystem.

Thematic Areas

Adaptation

Sperm competition intensity drives ejaculate performance mediated by seminal fluid in the black goby *Gobius niger*

Lisa Locatello, Oliviero Borgheresi, Andrea Pilaastro, Maria B. Rasotto

Department of Biology, University of Padova, Padova, Italy

Aim

Theory predicts male strategic allocation of resources to the ejaculate in response to the intensity of sperm competition. However, empirical tests are sparse and focused on sperm, overlooking the strategic allocation on the seminal fluid component of the ejaculate. The black goby, *Gobius niger*, an external fertilizing fish with guard-sneaker mating tactics, is an ideal model to fill this gap. Territorial males release ejaculates rich in viscous seminal fluid embedding sperm that slowly dissolve during spawning. Sneaker ejaculates contain 10-fold more sperm than those of territorials and 10-fold less seminal fluid, for a rapid and conspicuous sperm release. Sneaker males but not territorials, tailor the number of sperm released to the intensity of sperm competition. Here we test whether territorial males strategically adjust their ejaculate performance by exploiting seminal fluid.

Methods

Territorial males were exposed to different levels of sperm competition (absence of competing sneakers, 1 or 3 sneakers). Their ejaculate was manually stripped on a piece of acetate sheet and let to dissolve in sea water. The proportion of sperm released in 30 minutes over the total amount of sperm in the ejaculate was, then, estimated.

Results

The total number of sperm per ejaculate did not differ among treatments, but we found that a higher proportion of sperm was released in presence of 1 sneaker as compared to control (sneaker absent). However, when competition was more intense (3 sneakers) the proportion of sperm released was maintained lower, at the same level as in the no-competition treatment.

Main conclusion

Black goby territorial males appear to adjust their seminal fluid quality in relation to the perceived level of competition. A rapidly dissolving ejaculate or faster sperm might be advantage to defeat a competitor. By contrast, a slower dissolving ejaculate might allow to better face prolonged intrusions by consecutive sneakers.

Thematic Areas

Adaptation

Genes underlying light detection in *Hydra vulgaris*

Aide Macias-Muñoz^{1,2}, Rabi Murad¹, Ali Mortazavi¹

¹ Department of Developmental and Cell Biology, University of California, Irvine, CA, 92697 U.S.A.

² Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA, 93106 U.S.A.

Aim

Phototransduction is the signaling mechanism that underlies vision. The cascade has been described for two types of visual opsins: ciliary opsins common to vertebrates and rhabdomeric opsins common to arthropods. A third type of visual opsin is cnidop or cnidarian opsin whose cascade is not well known but are closely related to ciliary opsins. *Hydra vulgaris*, which lack eyes but exhibit light-dependent behavior, can be used to investigate candidate genes involved in cnidarian phototransduction.

Methods

We used reciprocal BLAST and gene ontology term annotations to identify candidate phototransduction genes in a reference genome and transcriptome. For opsin genes, we generated a phylogenetic tree that included other cnidarian species. We also used RNA-seq data from adult *Hydra* hypostome, tentacles, body column, foot, and budding zone in addition to time points in hypostome regeneration and during budding to quantify gene expression patterns.

Results

We identified a total of 45 opsin genes in *Hydra*. We determined that opsin genes are evolving by lineage-specific duplications and potentially by tandem duplications. We identified two opsin genes outside of the previously determined *Hydra* opsin groups; these genes possibly function in nematocytes and mucous gland cells, respectively. We found two opsin genes that have similar expression patterns to genes annotated with phototransduction functions.

Main Conclusion

We propose that the *H. vulgaris* phototransduction cascade could have similar components to both ciliary and rhabdomeric cascades.

Thematic Areas

Adaptation

Genetic variation analysis provides insights into diatom population genomics

Svenja Mager¹, Francesco Manfellotto¹, Maria Valeria Ruggiero¹, Viviana Di Tuccio¹, Monia Russo¹,
Lisa Campbell², Marina Montresor¹, Remo Sanges³, Mariella Ferrante¹.

¹Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

²Department of Oceanography, Texas A&M University, 3146 TAMU, College Station, TX 77843, USA

³Computational Genomics Laboratory, Neuroscience Area, International School for Advanced Studies (SISSA), Via Bonomea 265, 34136 Trieste, Italy

Aim

Diatoms form a major part of phytoplankton and play an important role in marine ecosystems. The project aims to shed light on population genomics by investigating genome regions which are more variable than others in different subgroups of strains of the planktonic diatom *Pseudo-nitzschia multistriata*.

Analysis of the variants in diverse *P. multistriata* genomes provides information about which genes are under selection or conserved between strains with the goal to deepen the understanding of genome evolution and adaptation for this species.

Methods

We resequenced the genome of 17 *P. multistriata* strains of different mating type (MT+ and MT-), collected at different locations and including two strains whose genotype dominated an atypical bloom in 2013. The variant information was used to produce phylogenetic trees to investigate relationships between the strains. Scaffolds diverging in the level of heterozygosity between groups of strains were identified. Fast evolving genes under selective pressure were identified by nonsynonymous to synonymous nucleotide diversity estimations.

Results

Preliminary analyses resulted in the finding of a mating-type specific scaffold in which MT- strains showed higher heterozygosity than MT+ strains. The two strains responsible for the clonal expansion event in 2013 were characterized by specific scaffolds diverging in heterozygosity levels from the other wild strains collected in the Gulf of Naples. Among the 17 strains, 57 genes were determined to be under positive selection.

Main Conclusion

Variant analyses in a diverse set of diatom strains provides a promising source for answering questions regarding genome evolution by allowing the determination of fast evolving genes and mutations that influence the behavior of groups of strains. Further analyses will include the determination of the functional categories of these genes, validation in additional strains and ultimately identification of the processes that drive functional differences between strains.

Thematic Areas

Populations and Species

Evolution and Biogeography of the Hydrozoa-Scleractinia symbiosis

Davide Maggioni^{1,2}, *Roberto Arrigoni*³, *Davide Seveso*^{1,2}, *Paolo Galli*^{1,2}, *Michael L. Berumen*⁴,
*Vianney Denis*⁵, *Bert W. Hoeksema*^{6,7}, *Danwei Huang*⁸, *Federica Manca*^{9,10}, *Daniela Pica*⁹, *Stefania*
*Puce*⁹, *James D. Reimer*^{10,11}, *Simone Montano*^{1,2}

¹ Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, 20126 Milan, Italy

² Marine Research and High Education (MaRHE) Center, University of Milano-Bicocca, 12030 Faafu Magoodhoo Island, Republic of Maldives

³ Department of Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn Napoli, Villa Comunale, 80121 Naples, Italy

⁴ Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

⁵ Institute of Oceanography, National Taiwan University, 10617 Taipei, Taiwan

⁶ Taxonomy and Systematics Group, Naturalis Biodiversity Center, 2300 RA Leiden, The Netherlands

⁷ Groningen Institute for Evolutionary Life Sciences, University of Groningen, 9700 CC Groningen, The Netherlands

⁸ Department of Biological Sciences and Tropical Marine Science Institute, National University of Singapore, 117558 Singapore, Singapore

⁹ Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona, Italy

¹⁰ Molecular Invertebrate Systematics and Ecology Laboratory, Graduate School of Engineering and Science, University of the Ryukyus, 903-0213 Okinawa, Japan

¹¹ Tropical Biosphere Research Center, University of the Ryukyus, 903-0213 Okinawa, Japan

Aim

Coral reefs are known to host the largest diversity of symbiotic associations in the marine environment. Scleractinian corals provide habitats for a broad variety of cryptofauna, which in turn may contribute to the overall functioning of coral symbiomes. Among these invertebrates, hydrozoans belonging to the genus *Zanclaea* represent an increasingly known and ecologically important group of coral symbionts.

Methods

In this study, about 300 *Zanclaea* colonies associated with scleractinian corals from the Indo-Pacific and Caribbean regions were analysed using an integrative approach. Specifically, molecular phylogenetics, species delimitation techniques, morphological analyses and comparative phylogenetic methods were used to shed light on the evolution and biogeography of the group.

Results

Overall, we found a high genetic diversity of hydrozoans despite their conserved morphology. However, preliminary results suggest that the obtained genetic clades may be successfully distinguished using statistical treatment of morphometric data and green fluorescence patterns, other than DNA data. The majority of clades were associated with one or two coral genera belonging to the Complex clade, whereas the remaining ones were generalists associated with both Complex and Robust corals. Despite the observed specificity patterns, no congruence between *Zanclaea* and coral phylogenies was observed, suggesting a lack of coevolutionary events. Most clades had a wide distribution across the Indo-Pacific, including a generalist one extending also in the Caribbean, while two host-specific clades were possibly endemics to the Red Sea, confirming the importance of this peripheral region as an endemism hotspot. Ancestral state reconstructions suggested that the most recent common ancestor of all extant coral-associated *Zanclaea* was a specialist species with a perisarc, occurring in what is now known as the Indo-Pacific.

Main conclusion

Ultimately, a mixture of geography- and host-related diversification processes is likely responsible for the observed enigmatic phylogenetic structure of coral-associated *Zanclaea*.

Thematic Areas

Populations and Species

Photosynthetic performance of the upside-down jellyfish *Cassiopea* sp.

Marta Mammone¹, Christine Ferrier-Pagés³, Silvia Lavorano⁴, Lucia Rizzo⁵, Stefano Piraino^{1,2}, Sergio Rossi^{1, 2, 6}

¹Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, DiSTeBA, University of Salento, Lecce, Italy

²CoNISMa, Consorzio Nazionale Interuniversitario per le Scienze del Mare, Rome, Italy

³Centre Scientifique de Monaco, Ecophysiology Team, 8 quai Antoine 1er, 98000 Monaco, France

⁴Acquario di Genova, Ponte Spinola, 16128 Genova, Italy

⁵Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, I-80121, Napoli, Italy

⁶Labomar, Universidade Federal do Ceará, Fortaleza, Brazil

Aim

Cassiopea andromeda is a mixotrophic Mediterranean alien species found in shallow water environments as lagoons and harbors. In the present study we investigated the short time reaction in the trophic strategy of *Cassiopea* jellyfish from a low irradiance to two different high irradiance conditions, simulating potential changes from eutrophic and turbid habitats to meso/oligotrophic waters. Understanding the physiological response mechanisms may allow to explain the adaptive potential of this non indigenous species in new habitats.

Methods

Jellyfish were at first acclimated to very low light condition and then moved for one week to two different higher light intensities. Photosynthesis measurements of *Cassiopea* sp. exposed to the two different PAR were carried out together with data obtained from PAM measurements. These data were complemented with measurements of symbiont density, chlorophyll a, and protein content to give a detailed frame of *Cassiopea* sp. autotrophic strategy.

Results

The short time response to different light treatment showed similar photosynthetic parameters in *Cassiopea* sp. The high photosynthetic rates were confirmed also by the RLCs. No significant differences between the two conditions were observed concerning symbiont abundance. However, jellyfish exposed to lower light intensity showed a higher content in chlorophyll a and c, and thus a higher Ci.

Main conclusion

No matter the acclimation at low irradiances, *Cassiopea* sp. showed high photosynthetic performances, demonstrating the ability of the species to adapt to fast changes in abiotic condition, such as light exposure. This ability, in addition to *Cassiopea* dual nutrition mode, makes this species a candidate survivor and potential winner in a changing oceans scenario against native species struggling these sudden changes.

Thematic area

Adaptation, Biodiversity

A pre-screening of the presence of antibiotics in traces in two large pelagic fish in the strait of Messina

Monique Mancuso^{ab}, Erika Arcadi^b, Danilo Malara^b, Marilena Sanfilippo^b, Pietro Battaglia^b, Franco Andaloro^b, Nunziacarla Spano^c, Teresa Romeo^b

^aInstitute for Marine Biological Resources and Biotechnology (IRBIM), National Research Country (CNR), Messina, Italy

^bStazione Zoologica Anton Dohrn, Centro interdipartimentale della Sicilia, Messina, Italy

^cUniversità degli Studi di Messina, Dipartimento di Scienze Biomediche, odontoiatriche e delle immagini morfologiche e funzionali, Messina, Italy.

Aim

The aim of our study was to carry out for the first time a pre-screening on the presence of antibiotics in commercial large pelagic fish, Mediterranean spearfish (*Tetrapturus belone*) and Bluefin tuna (*Thunnus thynnus*), collected in the Central Mediterranean Sea (Strait of Messina and surround area).

Methods

Specimens of *T. belone* and *T. thynnus* were catch by harpoon and hook respectively, during the fishing season 2019-2020 (in spring and summer). Muscle samples of (n. = 18 *T. belone*; n. = 7 *T. thynnus*) each fish specimen were took for the antibiotic screening performed by a rapid antibiotic test (Liofilchem). Two grams of each muscle samples were squeezed, diluted with distilled water (1:3 sample/water) homogenized, for 15 seconds and vortexed subsequently, the supernatant was withdrawn and 5 ml were inoculated in the test tube and incubated at 60°C for 6 h.

Results

From this first preliminary screening on a total of 25 fish, the results put in evidence show that these pelagic fish are not affected by the presence of antibiotics in muscle, in fact only a very low percentage (12%) of muscle samples was positive to antibiotics.

Main conclusion

Further analysis are needed, such as: a study based with HPLC to identify quantity and quality of each drug; an increasing of the number of analyzed fish; an extention of the study to sedentary species, or the searchtrace antibiotics in the water and sediments of the Strait of Messina in order to have a broader and more precise picture of this type of pollution.

Thematic Areas

Adaptation and Biodiversity.

Is it possible to predict the distribution of a rare parasitic species? The challenging case of *Savalia savaglia* (Bertoloni, 1819) (Cnidaria, Anthozoa)

Torcuato Pulido Mantas ^{a*}, Cecilia Varotti ^a, Marco Palma ^b, Mouloud Benabdi ^c, Egidio Trainito ^d, Vesna Macic ^e, Maria Cristina Gambi ^f, Carlo Cerrano ^a.

^a Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona 60131, Italy

^b UBICA srl (Underwater BIO-CARTography), Via San Siro 6 int.1, 16124 Genova, Italy

^c Laboratory Environmental Monitoring Network, University Oran 1, Ahmed Ben Bella, Oran, Algeria

^d Tavolara-Punta Coda Cavallo MPA, Via San Giovanni, 14-07026 Olbia, Italy

^e Institute of Marine Biology, University of Montenegro, P.O. Box 69, 85330 Kotor, Montenegro

^f Stazione Zoologica Anton Dohrn, Ischia Marine Centre, Punta San Pietro, 80077 Ischia, Italy

Aim

Despite the fact of being considered rare or endangered, many species miss effective monitoring measures or protection. This is the case of *Savalia savaglia* (Bertoloni, 1819), a parasitic zoanthid listed as “Near Threatened” by IUCN and included in the Annex II of the SPA/BIO Protocol of the Barcelona Convention. *S. savaglia* is an ecosystem engineer contributing to the biodiversity and the structural complexity of mesophotic benthic assemblages. Owing to its quite deep distribution, records of its presence are increasing only recently, thanks to ROV and technical diving explorations. On the present work we update its distribution and provide a predictive model to address eventual ground truthing surveys.

Methods

SDMs have been applied on the occurrences of *S. savaglia* obtained from different sources using bioclimatic variables from Bio-Oracle dataset as covariates. Presence only and presence/absence data were modelled using different algorithms (MaxENT, GAMs, RF, GLM). Model's predictions have been tested using cross-validation and their performance measured and compared by different evaluation metrics: AUC, TSS and environmental consistency. To finally average the more performant models in an ensemble for both presence only and presence/absence data.

Results

Considering the overall performances of the SDMs, better predictive powers were obtained using models developed with presence-only data, specially by random forest. Lower performances were obtained using presence-absence data which could be explained by the inconspicuous distributions of the species along with the fact that its parasitic behaviour was not considered by these first pool of SDMs.

Main conclusion

The case study of *Savalia savaglia* is particularly intriguing owing to its parasitic habitus. SDM is suggested as a promising tool identifying potential areas for future field investigations and helping on the process to protect *S. savaglia* populations from possible threats. Present results can provide a useful reference for predictive model distribution on rare species at large scale.

Thematic Areas

Biodiversity, Populations and Species

DNA from historical tiger shark (*Galeocerdo cuvier*) jaws suggests the disappearance of a local population off southeastern Australia

Alice Manuzzi^{1*}, Belen Jiménez-Mena¹, Romina Henriques¹, Bonnie J. Holmes², Janette Edson³, Mike Bennett⁴, Charlie Huveneers⁵, Jennifer R. Ovenden⁶, Einar Eg Nielsen¹⁻⁴

1 National Institute of Aquatic Resources, Technical University of Denmark, Vejlssøvej 39, 8600 Silkeborg, Denmark

2 School of Science & Engineering, University of the Sunshine Coast, Fraser Coast Campus, Hervey Bay, Queensland 4655, Australia

3 Queensland Brain Institute, University of Queensland, St Lucia Campus, Brisbane, Queensland 4072, Australia

4 School of Biomedical Sciences, University of Queensland, St Lucia Campus, Brisbane, Queensland 4072, Australia

5 Biological Sciences, Flinders University, Adelaide 5001, South Australia

6 Molecular Fisheries Laboratory, School of Biomedical Sciences, University of Queensland, St Lucia Campus, Brisbane, Queensland 4072, Australia

Aim

In the last century, species across the globe have experienced the effects of environmental and anthropogenic changes, and genetic analyses of historical collections represent a unique resource for tracking their impact on the genetic make-up of species. In this study we used a retrospective genomic approach to study the population of tiger sharks (*Galeocerdo cuvier*) in Eastern Australia. Due to the high mobility of the tiger shark, which is a globally distributed apex predator, we expected to find a single panmictic population at such local scale and as previously reported, but we were alert to the possibility of significant changes to the population over the last century as a consequence of documented population reductions.

Methods

The dataset used consisted of 116 unique samples originated from northeastern and eastern Australia, extending from the Gulf of Carpentaria to the Tasman Sea, and that were caught over a time-span of 80 years (1939-2015). Both the historical (jaws and vertebrae) and contemporary (fin clips) samples were subject to DNA capture using 20,000 gene associated “baits” from which ~2000 validated SNPs were successfully genotyped.

Results

Overall, we documented a significant change in genetic composition of tiger sharks over time and space. Our data is most compatible with a hypothesis of two distinct population groups, with varying contributions to spatiotemporal samples, and a population almost completely absent from contemporary and most northern samples. Findings that are possibly associated with differences in direct or indirect exploitation rates.

Main conclusion

This study shows that archived samples used in combination with modern genomic tools, can reveal temporal changes in biodiversity, which otherwise would have remained unnoticed. Such results highlights the importance of using genomic data to comprehend past evolutionary responses, as a key tool to inform decisions for the protection and management of elasmobranchs' populations.

Thematic areas

Populations and Species

Evolution of the AP-1 transcriptional complex: identification of novel components in the ascidia *Ciona robusta*

Pina Marotta¹, Federica Salatiello², Luca Ambrosino³, Federica Berruto², Maria Luisa Chiusano^{3,4},
Annamaria Locascio²

¹Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, Villa Comunale, 80121 Naples, Italy.

²Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms, Villa Comunale, 80121 Naples, Italy.

³Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.

⁴Department of Agriculture, Università degli Studi di Napoli Federico II, Portici, Italy.

Aim

The complexity of an organism correlates with the number of transcription factor (TF) in its genome; furthermore, their dimerization leads to a wider number of regulatory mechanisms. To understand how animals evolved, it is important to delineate the evolutionary mechanisms of these dimerizing TFs.

We focused on Jun and Fos, dimerizing TFs belonging to the bZIP proteins, representing the more diffuse components of the AP-1 complex in eukaryotes. The aim is to provide evidence of the Jun/Fos ability to form heterodimers and of their role during embryonic development of the ascidian *Ciona robusta*.

Methods

We scanned the *C. robusta* genome to identify bZIP members related to Jun and Fos, and performed a comparative approach by the use of different similarity thresholds to reconstruct homology networks involving Jun and Fos paralogs. Therefore, we analyzed their embryonic expression patterns and investigated the role of Jun and Fos in the *Ciona* notochord development. In this regard, the effects of their notochord-specific over-expression were evaluated.

Results

We detected 3 gene families of bZIP members related to JUN and FOS divided into 5 sub-families. By whole mount in situ hybridization (WMISH), we demonstrate that all these genes are detectable in the embryonic mesenchyme, with slightly different timing and localizations.

The co-overexpression of Jun and Fos in the notochord cells results in an abnormal embryonic phenotype, in which the main embryonic tissues (endoderm, muscles, mesenchyme and notochord) are properly differentiated, though a high grade of disorganization was detected in the notochord.

Main conclusion

The comparative analysis highlighted the presence of one JUN protein separated from 2 ATF2 isoforms, 4 isoforms encoded by FOS gene, 4 ATF3 isoforms and one MAF protein.

We demonstrate the involvement of a Jun/Fos heterodimer in the transcriptional regulation of developmental genes, giving a demonstration of their interaction in ascidia.

Thematic Areas

Macroevolution, Populations and Species.

Biases in demographic modelling affect our understanding of the process of speciation

Paolo Momigliano¹, Ann-Brit Florin² and Juha Merilä¹

¹Ecological Genetics Research Unit, Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

²Department of Aquatic Resources, Institute of Coastal Research, Swedish University of Agricultural Sciences, Öregrund, Sweden

Aim

Estimating patterns of gene flow during the early stages of speciation is central to understanding whether reproductive isolation arises through gradual erosion of gene flow or via successive stages of strict isolation and secondary contact.

Methods

Such scenarios can be tested by comparing the joint allele frequency spectrum (jAFS) of a set of populations to jAFS simulated under scenarios of isolation with migration (IM) and secondary contact (SC). However, the potential effect of unaccounted demographic events (such as population expansions and bottlenecks) on model choice and parameter estimation remains largely unexplored.

Results

Using simulations, we demonstrate that under realistic divergence scenarios with constant gene flow, failure to account for population size (N_e) changes in daughter population and ancestral populations leads to overestimate divergence time and to a bias towards the choice of SC models. On the other hand, when the simulations included long periods of strict isolation the correct gene flow scenario was usually retrieved. We illustrate these issues reconstructing the demographic history of North Sea and Baltic Sea turbot *Schophthalmus maximus* by testing 16 IM and 16 SC scenarios, modelling changes in N_e as well as the effects of linked selection and barrier loci. As with the simulations, failure to account for changes in N_e resulted in selecting SC models with long periods of isolation and divergence times preceding the formation of the Baltic Sea. In contrast, models accounting for N_e changes suggest that the Baltic Sea turbot population originated from a recent (<6 kya) invasion and has diverged with constant gene flow from the North Sea.

Main conclusion

The results have broad implications for the study of speciation by high-lighting the potential effects of ancestral size changes and bottlenecks followed by growth on choices between competing scenarios of speciation. In general, extreme caution should be exercised when interpreting results of demographic mode.

Thematic Areas

Adaptation, Populations and Species

***Cassiopea* Ephyra Development Across Separate Feeding Regimes**

Kaden Muffett

Texas A&M University

Aim

The past five years has seen a rise in the use of the genus *Cassiopea* as a scyphozoan model organism. Studies have explored behavior and environmental parameter tolerance limits of adults and strobilation of polyps, but there is currently no account of normal *Cassiopea* development under regulated conditions in a lab setting (35ppt salinity, 7mg/L O₂, 25°C).

Methods

To address this gap in the research, we have taken developmental images and size metrics of 50 *Cassiopea* from day of strobilation to the 42nd day across a variety of feeding regimes (0 kcal/day, 9.4×10^{-6} kcal/day, 18.8×10^{-6} kcal/day, 37.5×10^{-6} kcal/day) to establish a growth curve and mortality in a highly regulated, low PAR environment.

Results

All *Cassiopea* feeding treatment groups developed at consistent rates. While the unfed treatment group had the highest mortality (23%), surviving unfed individuals managed to remain stable in size across all 42 days with no visible transition away from their immediately post-strobila state. All fed groups experienced a significant increase in the mean oral arm length to bell radius ratio, while unfed medusae did not, however within fed medusae this ratio was not correlated with increased feeding amounts. At the end of 42 days, symbiont density was determined by ephyra homogenization and varied dramatically between individuals from 117 cells/g to 1139 cells/g, not well correlated with feeding regime. Unfed medusae retained more ephyra-like behaviors, spending less time settled on the bottom of containers.

Main conclusion

Together this suggests that unfed ephyrae can maintain size for up to 6 weeks and largely experience a pause in development of adult characteristics without feeding.

Thematic Areas

Adaptation

Different fates of seagrasses in an era of ocean warming

Hung Manh Nguyen¹, Peter J. Ralph³, Lázaro Marín-Guirao^{1,2§}, Mathieu Pernice^{3§} and Gabriele Procaccini^{1§}

¹Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.

²Seagrass Ecology Group, Oceanographic Centre of Murcia, Spanish Institute of Oceanography, C/ Varadero, s30740 San Pedro del Pinatar, Murcia, Spain.

³University of Technology Sydney, Faculty of Science, Climate Change Cluster (C3), Sydney, New South Wales 2007, Australia.

[§] Authors contributed equally to this work

Aim

Seagrasses are a unique group of angiosperms that has evolutionarily recolonized the marine environment some 60-90 mya. Seagrasses exist across thousands of kilometres of the sedimentary shorelines where they provide one of the most valuable sources of food and habitat for marine life, together with being one of Earth's most efficient carbon sinks with direct effects on mitigating global warming. Nonetheless, seagrasses are negatively being pushed into a rapid evolution driven by both natural and anthropogenic stressors with ocean warming being one of the major players. Recently, alongside the growing recognition of the importance of seagrasses and the recent application of advanced molecular technologies, the number of studies focusing on the interactions of warming and seagrasses has increased rapidly.

Methods

We have gathered information from published studies to identify potential commonalities in the effects of warming and the responses of seagrasses at four different levels: molecular, biochemical/physiological, morphological/population, and ecosystem/planetary. Warming strongly affect seagrasses at all four levels and alters seagrass distribution by causing massive die-offs in some seagrass populations, whilst also enhancing tropicalization and migration of temperate species.

Results

We conclude by discussing the most significant knowledge gaps and future directions for seagrass research. Our comprehensive view about seagrasses in an era of ocean warming not only applies to seagrass scientific and management community but also provides a set of useful information for addressing the response capability of marine organisms in general and to keystone species in particular, such as corals, mangroves, and seaweeds. Furthermore, we hope to encourages local stakeholders and decision-makers on designing appropriate strategies for protecting seagrasses as well as raise public awareness on the importance of seagrass protection and restoration for a sustainable future.

Thematic Areas

Populations and Species

The past, present and future of cleaner fish cognitive performance as a function of CO₂ levels

José Ricardo Paula¹, Miguel Baptista¹, Francisco Carvalho¹, Tiago Repolho¹, Redouan Bshary², Rui Rosa¹

¹MARE—Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Av. Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal

²Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

Aim

Ocean acidification is one of the many consequences of climate change. Various studies suggest that marine organisms' behaviour will be impaired under high CO₂.

Methods

Here, we show that the cognitive performance of the cleaner wrasse, *Labroides dimidiatus*, has not suffered from the increase of CO₂ from pre-industrial levels to today, and that the standing variation in CO₂ tolerance offers potential for adaptation to at least 750 μ atm. We acclimated cleaners over 30 days to five levels of pCO₂, from pre-industrial to high future CO₂ scenarios, before testing them in an ecologically relevant task—the ability to learn to prioritise an ephemeral food source over a permanent one.

Results

Fish learning abilities remained stable from pre-industrial to present-day pCO₂. While performance was reduced under mid (750 μ atm) and high CO₂ (980 μ atm) scenarios, under the former 36% of cleaners still solved the task.

Main conclusion

The presence of tolerant individuals reveals the potential for adaptation, as long as selection pressure on cognitive performance is strong. However, the apparent absence of high CO₂ tolerant fish, and potentially synergistic effects between various climate change stressors, renders the probability of further adaptation unlikely.

Thematic Areas

Adaptation; Biodiversity

Transcriptional responses of *Posidonia oceanica* under multiple stresses: the influence of the native environment

Jessica Pazzaglia^{1,2}, Alex Santillán-Sarmiento¹, Miriam Ruocco¹, Emanuela Dattolo¹, Antonio Terlizzi², Lazaro Marín-Guirao³, Gabriele Procaccini¹

¹ Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, 80121, Italy

² Department of Life Sciences, University of Trieste, 34127, Trieste, Italy

³ Seagrass Ecology Group, Oceanographic Center of Murcia, Spanish Institute of Oceanography, C/ Varadero, 30740 San Pedro del Pinatar, Spain.

Aim

Seagrasses are particularly susceptible to environmental changes such as the intensification of warming and eutrophication events. The occurrence of rapid environmental changes are threatening natural populations.

Posidonia oceanica is an endemic Mediterranean species and ranks amongst the slowest- growing and longest-lived plants on earth. Recent evidence revealed that *P. oceanica* plants distributed along bathymetric or latitudinal gradients show different transcriptomic responses to common stress, suggesting a crucial role of pre- adaptation to local environmental conditions in driving response to stress. Here we aimed to analyze the physiological and transcriptional responses of *P. oceanica* plants with different life histories to multiple stresses.

Methods

P. oceanica shoots collected from sites with different nutrient conditions (Eu plants = Eutrophic plants; Ol Plants = Oligotrophic plants) were exposed in a common garden experiment to nutrient enrichment, temperature increase and their combination. Leaf tissue was collected after two weeks of exposure to stressed conditions to assess the physiological status of plants and to analyze gene expression profiles, applying the TAG sequencing technique.

Results

Plants showed different transcriptional profiles against the same stress conditions. Differentially expressed genes varied among plants, with a higher genes activation in plants growing in a more stressed environment, in respect to plants growing in a pristine environment. The effect of temperature at physiological level was lower in comparison to nutrients and the analysis, as confirmed by gene expression results. An interesting expression pattern of methylation processes was observed, including a large number of genes related to histone modifications, supporting their role in modulation and control of gene expression.

Conclusion

The different life history of *P. oceanica* plants is crucial to understand future persistence of this species under rapid environmental changes. Local environmental conditions seem to modify plant responses to multiple stresses through transcriptional regulation, which could have an epigenetic basis.

Thematic Areas

Adaptation, Populations and Species

Natural selection in the evolutionary divergence between two penguin species

Federica Pirri^{†1}, *Lino Ometto*^{‡2}, *Silvia Fuselli*^{§3}, *Lorenzo Zane*^{†4}, *Emiliano Trucchi*^{*5}

[†]Department of Biology, University of Padova

[‡]Department of Biology and Biotechnology, University of Pavia

[§]Department of Life Sciences and Biotechnology, University of Ferrara

^{*}Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona

Aim

Which are the genetic bases of the adaptation of penguins to extreme temperature conditions? To address this question, we plan a comparative genomic study between the Emperor penguin (*Aptenodytes forsteri*), that breeds only in Antarctica, and its much less cold-adapted sibling species, the King penguin (*A. patagonicus*), that reproduces exclusively in ice-free sub-Antarctic islands. We aim to detect signatures of differential selection as well as to reveal genetic traits relevant to adaptation to cold in the Emperor penguin.

Methods

To identify candidate genes involved in lineage-specific adaptations, we first implemented a selection analysis using the ratio of non-synonymous and synonymous variation (ω) comparing the orthologous genes across several penguin and bird species.

Then we ran a haplotype-based test (XP-EHH) using population-level genomic data to discover more recent and less intense selection events that were mostly specific to each species.

Finally, to investigate the functions of the candidate genes for positive selection, we performed a GO terms overrepresentation analysis.

Results

The first analysis revealed strong evidence of differential selection for 68 genes indicating that, for these genes, either the King or the Emperor was indeed evolving at different rates compared to 7 penguin and 13 other bird species in the examined phylogeny.

A higher number of candidate SNPs and genes under positive selection was detected in the Emperor penguin by both statistical methods and most of them were related to metabolism, response to stimulus, development and signaling according to GO terms enrichment analysis.

Main conclusion

Our study showed that the Emperor penguin has been subject to more pervasive selection pressures than the King and that several candidate genes which exhibited signals of positive selection in the Emperor penguin were associated with processes that are relevant for adaptive evolution in the extreme Antarctic conditions.

Thematic Areas

Adaptation, Populations and Species

Switches, stability and reversals: the evolutionary history of sexual systems in fish

Susanna Pla¹, Isabella Capellini^{2}, Chiara Benvenuto^{3*}, Francesc Piferrer^{1*}*

¹Institute of Marine Sciences, Spanish National Research Council (CSIC), Barcelona, Spain

²School of Biological Sciences, Queen's University Belfast (UK)

³School of Science, Engineering and Environment, University of Salford, Salford (UK)

Aims

Most organisms reproduce sexually and individuals maintain a fixed sex throughout their life (gonochorism). In some species, however, individuals can produce male and female gametes at the same time (simultaneous hermaphroditism) or reproduce initially as one sex and later in life switch to the other (sequential hermaphroditism, with female being the first sex in protogyny and male the first sex in protandry). Theoretical models, initially developed for plants, suggest that gonochorism and simultaneous hermaphroditism are evolutionary stable conditions, but if this is the case in animals is unclear, especially in sexually plastic taxa such as teleosts.

Methods and results

Using Bayesian phylogenetic comparative methods and 4740 teleost species, we show that gonochorism is likely to be the ancestral condition from which protogyny, protandry and simultaneous hermaphroditism evolve at a moderate evolutionary rate. While all forms of hermaphroditism revert as quickly or faster to gonochorism, protogyny and simultaneous hermaphroditism are evolutionarily more stable than protandry, as the latter is quickly lost to other sexual systems. Moreover, we report for the first time a direct pathway from gonochorism to simultaneous hermaphroditism, without the need of intermediary evolutionary transitions (e.g., androdioecy or sequential hermaphroditism). Finally, we expanded the theoretical framework of the evolution of hermaphroditism investigating how life history traits change among sexual systems: as predicted, protogynous fish live longer, allowing individuals to take advantage of the reproductive value of dominant males, while protandrous species are bigger, maximizing the fecundity of females.

Main conclusions

While including life history traits is important, it is not sufficient to explain the distribution of hermaphroditism among teleosts (Williams' paradox). We propose the use of a comprehensive framework, which includes mating systems, sex determining mechanisms and developmental gonadal plasticity, to gather a better understanding of the unique evolution and distribution of hermaphroditism in the largest radiation of vertebrates.

Thematic Areas

Macroevolution, Populations and Species

Exploring variations in personality and morphometric traits in wild juveniles of sand smelt captured in different environments and with different fishing techniques in the Venetian Lagoon

Federica Poli¹, Giovanni Polverino², Pietro Antolini¹, Alberto Nóvoa Abelleira¹, Carlotta Mazzoldi¹

¹University of Padova, Italy

²The University of Western Australia

Aim

The pace-of-life syndrome (POLS) hypothesis posits consistent variation in behavior among individuals (personality) to covary with variation in life history traits. Within species, the relationship among these traits is expected to plastically respond to changes in ecological conditions. Trade-off should vary along environmental gradients, but the extent to which relationships between POLS traits vary across environmental conditions remain unclear.

Methods

Here, we measured consistent among-individual variation in both behavior (personality) and morphology (body shape) within and between groups of juveniles of the big-scale sand smelt (*Atherina boyeri*) - a fishery-target species in the Venetian Lagoon. We compared individuals caught in environmentally different areas and individuals caught with active or passive fishing techniques.

Results

We suggest that among-individual variation in behavior and morphology may emerge in juveniles growing in different conditions. In particular, the chosen areas differ in their distance from the sea harbor (more/less exposed to lagoon-sea water exchange) and in the habitat complexity (seagrass meadows vs sandbanks). Second, some personality, such as boldness or high swimming activity, morphological traits may influence juveniles' vulnerability to fishing.

Main conclusion

Such plastic responses in early developmental stages may be critical in a scenario of environmental changes and fishery induced evolution.

Thematic Areas

Populations and Species

Illuminating adaptive divergence (and speciation?) with gene flow through hybrid zones in the marine snail *Littorina saxatilis*

Francesca Raffini^{1,2}, Anja Westram^{1,3}, Rui Faria^{1,4}, Sean Stankowski¹, Juan Galindo⁵, Roger Butlin¹

¹ The University of Sheffield, Sheffield, United Kingdom

² University of Ferrara, Ferrara, Italy

³ Institute of Science and Technology Austria, Klosterneuburg, Austria

⁴ CIBIO-InBIO. Research Center in Biodiversity and Genetic Resources
University of Porto, Porto, Portugal

⁵ University of Vigo, Vigo, Spain

Aim

Adaptation and speciation are key processes that determine biological diversity and its distribution in space and time. Speciation requires the evolution of barriers to gene flow between diverging populations. The mechanisms underlying these processes are often unclear, particularly when they occur in the face of gene flow. An ideal model to understand the factors enabling adaptive divergence with gene flow is the marine snail *Littorina saxatilis*. It shows repeated adaptive divergence between crab-adapted and wave-adapted ecotypes. They are ecologically, morphologically and behaviourally distinct but hybridize in the contact zones.

Methods

Here, we take advantage of these hybrid zones to uncover the genomic architecture of parallel adaptive divergence with gene flow in Sweden and Spain, the extremes of *L. saxatilis* distribution in Europe.

Results

Our preliminary analyses of crab-hybrid-wave transects using low coverage whole genome resequencing showed clinal variation in Sweden but two discrete groups in Spain. This result suggested that crab and wave are at a more advanced stage of speciation at the southern end. By studying both locations, we are able to understand how reproductive isolation emerge and population diverge. Notably, genomic divergence between crab and wave do not fully correspond to habitat transition in both countries. This pattern indicates that ecologically diversifying selection might not be the single evolutionary force at play in *L. saxatilis*' adaptive polymorphism. Further analyses including experimental fieldwork and ecological niche modelling will clarify the relative role of environmental and genetic mechanisms in a natural system such as *L. saxatilis*, which is crucial to obtain a more complete and comprehensive understanding of the mechanisms underlying (adaptive) divergence.

Main conclusion

Our findings will shed light into the factors and interactions that promote speciation-with-gene-flow in nature.

Thematic Areas

Adaptation, Biodiversity

Mutualistic cleaning behavior between two coral reef fish species: a molecular approach

Ramírez-Calero, S*, Paula, J. R[‡], Cascalheira, L[‡], Rosa, R[‡], Ravasi, T[§], Schunter C*

*School of Biological Sciences, Swire Institute of Marine Science. Kadoorie Biological Sciences Building, The University of Hong Kong, Hong Kong, Hong Kong, SAR

‡MARE – Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia – Faculdade de Ciências da Universidade de Lisboa, Av. Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal

§Marine Climate Change Unit, Okinawa Institute of Science and Technology Graduate University (OIST), 1919-1 Tancha, Onna-son, Okinawa, 904-0495 Japan

Aim

Social interactions are fundamental in nature, but little is known about the neural mechanisms establishing the social relationships between two species of reef fish. Marine fishes offer a great opportunity to understand these interactions as some species depend on others through symbiotic relationships. In particular, the cleaner fish *Labroides dimidiatus* allows us to study mutualistic cleaning interactions displaying sophisticated social behavior while interacting with other species known as ‘clients’, that can benefit from ectoparasite reduction and stress relief.

Methods

To understand this essential inter-specific behavior, this study identified using transcriptomics, relevant molecular pathways related to social behavior in the fore-, mid- and hindbrain regions of the cleaner fish and one of its potential clients (*Acanthurus leucosternon*).

Results

Comparing the brains of interacting fish vs fish alone in aquariums, we found that the social interaction of *L. dimidiatus* with its client fish involved several social neural systems such as the dopaminergic, glutamatergic and GABAergic pathways as well as social decision-making genes. In addition, we found immediate early gene responses related to social plasticity and transcription factor activity such as *c-jun*, *c-fos* and CREB indicating neural activation during interaction. The client fish on the other hand had less differential gene expression across the brain and genes were functionally involved in hormonal activity. For both species most of the neural activation during interaction was produced and regulated in the hindbrain and forebrain region.

Main conclusions

These results suggest an important implication of hormone systems and the activation of immediate early genes during the social interaction of *L. dimidiatus* and *A. leucosternon*. Analysing the role of these molecular mechanisms in the brain provides key information on crucial inter-specific behaviours and can shed light on the key mechanisms of vertebrate mutualism among marine species.

Thematic areas

Biodiversity, Populations and species

DNA metabarcoding unveils dietary niche partitioning in the Adriatic European hake and support fisheries management

Giulia Riccioni^{*1}, *Marco Stagioni*^{1,2}, *Chiara Manfredi*^{1,2}, *Fausto Tinti*¹, *Corrado Piccinetti*¹, *Simone Libralato*³

¹ Department of Biological, Geological and Environmental Sciences, University of Bologna, Piazza di Porta S. Donato, 1, 40126 Bologna, Italy.

² Co.N.I.S.Ma- URL Fano, Laboratory of Marine Biology and Fishery, viale Adriatico 1/n, 61032 Fano, Italy

³ National Institute of Oceanography and Applied Geophysics - OGS, Via Beirut 2/4 (Ex-Sissa building), 34151 Trieste, Italy.

*Current Address: Fondazione Mach-Istituto Agrario di S. Michele all'Adige, Research and Innovation Centre (CRI), Via E. Mach, 1 - I-38010, S. Michele all'Adige (Trento), Italy

Aim

The Northern-Central Adriatic Sea is one of the most productive areas of the Mediterranean Sea including a broad diversity of habitats. Within this basin, the Pomo Pit is one of the most important areas of aggregation and is an important spawning/nursery area in the Northern and Central Adriatic for the stock of European hake (*Merluccius merluccius*), a species of relevant commercial value heavily exploited by fishing activities. In this study we applied a metabarcoding approach to study the feeding habits of European hakes sampled within the Pomo Pit and to compare this feeding behaviour to the diet of hakes caught outside the Pomo area.

Methods

European hake specimens were collected in 2016, between 46 to 260 m depth along the coast of the Adriatic Sea from the Gulf of Trieste to Pomo/Jabuka pit. Ten individuals each of the five size classes in the two different habitat (shallow water outside the Pomo Pit and deep water within the Pomo Pit) for a total of 100 individuals were selected for the metabarcoding analyses.

Results

Our metabarcoding analyses proved the presence of specific niche partitioning and food preferences between hakes living inside and outside the Pomo Pit confirming the ontogenetic shift from a diet based mainly on crustaceans for juveniles to a more piscivorous feeding behaviour for adult fish. These results for the first time highlighted the crucial role played by the Pomo Pit in *Merluccius merluccius* feeding behaviour and ontogenetic development strengthening the hypothesis of the presence of an Essential Fish Habitat and promoting a careful management of this ecosystem through focused conservation measures.

Main conclusions

These outcomes emphasize the need of updated scientific methods as metabarcoding or DNA-based methods to study marine food webs and integrate the current information about the feeding behaviour of marine organisms.

Thematic areas

Biodiversity, Populations and Species

Digging into bivalve miRNAomes: between conservation and innovation

Umberto Rosani^{a*}, Enrico Bortoletto^a, Chang-Ming Bai^b, Antonio Figueras^c, Paola Venier^a, Bastian Fromm^d

^aDepartment of Biology, University of Padova, 35121 Padova, Italy, ORCID ID

^bKey Laboratory of Maricultural Organism Disease Control, Ministry of Agriculture; Qingdao Key Laboratory of Mariculture Epidemiology and Biosecurity; Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266237, China

^cInstitute of Marine Research (IIM), Spanish National Research Council (CSIC). Eduardo Cabello, 6, 36208, Vigo, Spain

^dScience for Life Laboratory, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, S-10691 Stockholm, Sweden

Aim

Bivalves are a diverse mollusc group of economic and ecological importance. Their evolutionary resilience to pollution, parasites and extreme environments make them important model systems for studying adaptation and immunology. Despite substantial progress in sequencing projects of bivalves, this is currently contrasted with a lack of comparative genomics and transcriptomic datasets especially for non-coding and gene-regulatory aspects.

Methods

We here review the current state of bivalve microRNA complements, the most important and most conserved gene-regulators across Metazoa. We find substantial heterogeneity and incorrect annotations of microRNAs in the five available bivalve species that we corrected by reannotation of conserved microRNA families using recently established criteria for *bona fide* microRNA genes, and expanded it by adding two novel species, the Mediterranean mussel *Mytilus galloprovincialis* and the blood clam *Scapharca broughtonii*.

Results

Altogether we found 104 missing microRNA families in the previously published bivalve datasets and 89 families and 147 genes in the two novel species, respectively, that give a very homogeneous and consistent picture of regulatory evolution in bivalves and enable future comparative studies.

Main conclusion

By re-evaluating and re-annotating conserved miRNAs from previous reports and expanding miRNAome data to two additional species, we showed how incomplete previous annotations were and demonstrated a high conservation of miRNA families, according to the taxonomic position of bivalves. This not unexpected discovery opens up for novel paths to study evolution of bivalves with miRNAs and, *vice versa*, miRNA evolution in bivalves, as we have identified distinct patterns of miRNA expansion, putative losses and two bivalve-specific miRNA families. The identification of two bivalve specific microRNA families sheds further light into bivalve specific gene-regulatory adaptations and trail-blazes directions of bivalve research in the future.

Thematic Areas

Macroevolution, Adaptation.

The evolution of molecular toolkits for biosilicification in demosponges (Porifera)

Maria Eleonora Rossi^{1,2}, Nathan James Kenny³, Sergi Taboada⁴, Vasiliki Koutsouveli², Bruna Plese², Davide Pisani¹, Ana Riesgo²

¹ School of Earth Sciences, University of Bristol, Bristol BS8 1TH,

² Life Sciences Department, The Natural History Museum, London SW7 5BD, UK

³ Faculty of Health and Life Sciences, Oxford Brookes University, Oxford OX3 0BP, UK

⁴ Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Spain

Aim

Among the four classes of Porifera, three of them construct siliceous skeletons but through divergent enzymatic pathways. In demosponges, the silicification occurs by polycondensation of silica using silicases around an axial filament formed by a protein called silicatein. Most demosponges can produce more than one spicule type, in some occasions with convoluted ornamentations. Some demosponges have more than one silicatein gene (with highly divergent evolutionary pathways). In calcareous sponges, the many alpha-carbonic anhydrases present in the group are linked to the production of several spicule types. Whether the diversity of siliceous spicules is linked to larger molecular complexity in demosponges, is completely unknown.

Methods

Here we used complete transcriptomes of 72 sponges (19 newly assembled) to create a sound phylogenomic framework to explore the evolution of biosilicification within demosponges. The enzymes required to produce siliceous spicules including silicases, silicateins, and silicon transporters, were screened within our transcriptomic and other datasets available and their expression quantified using bowtie2/RSEM. Character reconstructions were performed in our datasets to understand the evolution of the molecular machinery. Finally, we used BAMM tools to detect and quantify heterogeneity in evolutionary rates across sponges with diverging silicification levels, in terms of number of spicule types and spicule total content.

Thematic Areas

Biodiversity, Macroevolution

Cryptic species complexes in the marine diatom *Pseudo-nitzschia* explored by metabarcoding

Ruggiero M.V.^a, Kooistra W.H.C.F.^a, Montresor M.^a, Piredda R.^a, Sarno D.^b, Zingone A.^a

^a Integrative Marine Ecology - Stazione Zoologica Anton Dohrn - Villa Comunale, 80121, Naples, Italy

^b RIMAR - Stazione Zoologica Anton Dohrn-Villa Comunale, 80121, Naples, Italy

Aim

Pseudo-nitzschia is a cosmopolitan marine diatom genus. It encompasses more than 50 species, with several cases of cryptic and pseudo-cryptic species. Many of the taxa are toxic, so it is important to correctly identify them. Here we used meta-barcoding data collected at the LTER Station MareChiara in the Gulf of Naples to explore the genetic structure of the genus.

Methods

We used the V4-region of the 18S rDNA as reference to retrieve *Pseudo-nitzschia* reads from 48 environmental DNA samples collected along three years. The reference sequences were used as queries in blast analysis, phylogenetic building and SWARM clustering to assign metabarcode haplotypes to species. Haplotype frequency distributions, haplotypic and nucleotidic diversity and temporal patterns of occurrence were analysed within each species. Median-joining networks and sequence divergence were used to assess interspecific relationships within and among cryptic species complexes.

Results

The V4 barcode showed good resolution power in most cases.

Haplotype assignment to known species was usually straightforward. Signs of incomplete lineage sorting and/or introgression between species were nevertheless observed within the “*delicatissima*” and the “*galaxiae*” species-complex. Moreover, a highly abundant, intermediate haplotype between two of the “*delicatissima*” species was found. The frequency spectrum of the haplotypes showed a highly abundant haplotype and many low frequency ones within single taxa. Values of mean distance between taxa were different among species complexes. Almost all taxa showed a regular seasonal pattern among years.

Conclusions

Metabarcoding possesses a high resolution, allowing a deeper look on the relationships within and between species complexes.

Cryptic species occur in offset or different periods, suggesting ecophysiological differentiation, which could be used in combination with blast analysis for a correct assignment of the reads.

Signs of incomplete lineage sorting and/or introgression between species suggest lingering hybridization events.

Thematic Areas

Populations and species, Biodiversity

m⁶A RNA methylation in seagrasses: first insights and relevance for biological rhythms

*Miriam Ruocco**, *Luca Ambrosino**, *Marlene Jahnke†*, *Maria Luisa Chiusano*§*, *Isabel Barrote‡*,
*Gabriele Procaccini**, *João Silva‡*, *Emanuela Dattolo**

¹ Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

†Department of Marine Sciences, University of Gothenburg Lovén Centre - Tjärnö 45296 Strömstad, Sweden

§ Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (Na), Italy

‡ CCMar-Centre of Marine Sciences, University of Algarve, Campus of Gambelas, 8005-139 Faro, Portugal

Aim

One fundamental aspect of environmental adaptation is the synchronic adjustment of physiological activities with environmental cues driven by the circadian-clock system. Recent studies have shown that epigenetic and epitranscriptomic mechanisms (e.g. RNA methylation) regulate the circadian clock in several organisms, but only few studies have focused on plants. Methylation of RNA bases (m⁶A) is the most prevalent modification, carried out by proteins responsible of adding (*writers*) and deleting (*erasers*) methylation marks. Here, we aimed at characterizing m⁶A-related genes in seagrasses and get insights into their role in regulating circadian rhythms.

Methods

We provided a first inventory of m⁶A-related genes in two seagrass species (*Cymodocea nodosa* and *Zostera marina*) exploiting available genome and transcriptome data and comparing the results with terrestrial model plants. We further investigated daily changes in global m⁶A RNA methylation and transcript levels of writers and erasers genes over a 24h cycle, across species and latitudes, via RT-qPCR.

Results

Z. marina and *C. nodosa* possess the entire repertoire of m⁶A genes, with an expansion of a writer (*FIP37*) gene family. *C. nodosa* was redundant in transcripts encoding for erasers. Our analyses also revealed a striking rhythmicity of writer and eraser genes and concomitant m⁶A levels in both species. M⁶A methylation always peaked toward the dark period, suggesting a role in driving transcriptome rearrangements during the night. In general, the observed patterns were asynchronous between species at the same location, while being very similar in populations growing at contrasting latitudes, suggesting a regulation by endogenous species-specific cues more than by external stimulus.

Main conclusion

RNA methylation could widely participate in the daily regulation of gene expression and circadian-clock functions in seagrasses, potentially affecting the photo-biological behaviour of the species and their ability to spread across different latitudes and photoperiodic regimes.

Thematic Areas

Populations and Species, Adaptation

Mass mortality of *Pinna nobilis* in Sardinia: phylogenetic inference on the etiological agents.

Sanna D.¹, Scarpa F.², Azzena I.^{1,2}, Cossu P.², Casu M.²

¹Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy

²Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Via Vienna 2, 07100 Sassari, Italy

Aims

The fan mussel, *Pinna nobilis* represents the largest bivalve of the Mediterranean Sea, where it is an endemic flagship species. Unfortunately, since 2016 a dramatic mass mortality has been observed in several Mediterranean areas. Epidemiological surveys indicated as potential cause the co-occurrence of different etiological agents, (e.g. *Haplosporidium pinnae*, *Mycobacterium* sp., *Vibrio* spp. and *Perkinsus* spp.). Here we performed diagnostic molecular surveys on populations of *P. nobilis* from Sardinia to provide insights on phylogeny and taxonomic status of the possible etiological agents.

Methods

Diagnostic analyses have been performed on 48 samples of *P. nobilis* and two of the congeneric *P. rudis*, collected in Sardinia recently, to obtain 18S and 16S ribosomal DNA sequences of the protozoa and bacteria infecting fan mussel tissues.

Results

Twenty-seven identical sequences of *H. pinnae* (18S rDNA) were obtained. All these sequences corresponded exactly to one of those already available on GenBank, therefore no phylogenetic surveys were performed. Four sequences of the 16S rDNA of *Mycobacterium* sp. were isolated from *P. nobilis* and *P. rudis*. Phylogenetic analyses showed that *Mycobacterium* spp. found in our samples are closely related to common mycobacteria and belong to three never described before taxonomic entities.

Main conclusion

Our findings indicate the occurrence of several species of *Mycobacterium* in the Sardinian marine environment, although they might not play a pathogenic role for fan mussels. Indeed, we isolated sequences of mycobacteria from both dead and healthy individuals of *P. nobilis* and *P. rudis*, suggesting that their presence may not be necessarily linked to the causes of the mass mortality. The large number of mycobacteria taxonomic entities found likely infecting the fan mussel, suggests that their overall role in the mass mortality of *P. nobilis* should be re-evaluated and support the occurrence of a multifactorial disease as a possible explanation of this event.

Thematic Areas

Populations and Species, Biodiversity.

Invasions of deep-sea and freshwater habitats increase diversification rates in anguilliform fishes

*Francesco Santini**

*Associazione Italiana per lo Studio della Biodiversità, 58100, Grosseto, Italy

Aim

Anguilliform fishes are an ecologically diverse clade distributed in all marine habitats, from deep sea trenches to oceanic pelagic ecosystems, from tropical coral reefs to coastal sandy bottoms. Recent molecular phylogenies have dramatically changed our understanding of the relationships among major eel lineages, and provided us with a better understanding of the timeline of eel evolution. Little is known, however, about how the repeated habitat shifts that have occurred within this group may have influenced evolutionary rates and contributed to the present diversity of eels.

Methods

I generated a new relaxed clock timetree for anguilliforms using a new molecular dataset (7 loci for 272 species), and used several phylogenetic comparative methods in combination with ecological and morphological data (habitat type, depth, body size) to test whether habitat shifts affected rates of diversification within anguilliforms.

Results

The new time-calibrated phylogeny suggests that eels likely originated in marine coastal habitats during the late Cretaceous, with several lineages independently invading deep sea environments during the Eocene or Oligocene. While multiple invasions of coral reef habitats did not produce significant shifts in the rate of lineage or body size diversification, habitat shifts towards the deep-sea led to dramatic increases in rates of lineage diversification compared to their shallow water relatives. The highest rates of both lineage and body size diversification, however, occur within the true eels (Anguillidae), the only group to have successfully colonized freshwater habitats.

Main conclusion

Habitat shifts towards deep-sea or freshwater environments appear to significantly increase the rate of lineage diversification in eels, while invasion of tropical coral reefs does not. True eels are a very young and diverse group that is likely experiencing an adaptive radiation

Thematic Areas

Macroevolution, Populations and Species

Size-selective mortality induces evolutionary changes in group risk-taking behavior and the circadian system in a fish

Valerio Sbragaglia^{1,2}, Jose Fernando López-Olmeda³, Elena Frigato⁴, Cristiano Bertolucci⁴, Robert Arlinghaus^{1,5}

¹ Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

² Department of Marine Renewable Resources, Institute of Marine Sciences (ICM-CSIC), Barcelona Spain

³ Department of Physiology, Faculty of Biology, Regional Campus of International Excellence “Campus Mare Nostrum”, University of Murcia, Murcia, Spain

⁴ Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

⁵ Division of Integrative Fisheries Management, Faculty of Life Sciences & Integrative Research Institute on Transformations of Human-Environment Systems (IRI THESys), Humboldt-Universität zu Berlin, Berlin, Germany

Aim

Size-selective mortality is a major evolutionary driver of life-history and behavioral traits in human-dominated marine ecosystems. These changes might in turn alter the circadian system and therefore affect daily physiological processes and behavioral outputs.

Methods

The proximate mechanisms governing evolution are difficult to disentangle in the wild. We overcome this limitation by studying the evolutionary impact of size-selective harvesting on group risk-taking behavior and the circadian system in a model fish species. We exposed zebrafish (*Danio rerio*) to either large or small size-selective harvesting relative to a control over five generations, followed by eight generations during which harvesting halted.

Results

Size-selective mortality affected fine-scale timing of behaviours. In particular, small size-selective mortality, typical of gape-limited predators targeting smaller size classes, increased group risk-taking behavior. Moreover, small size-selective mortality increased early peaks of daily activity as well as extended self-feeding daily activity to the photophase compared to controls. By contrast large size-selective mortality, typical of most wild capture fisheries, only showed an almost significant effect of decreasing group risk-taking behavior and no clear changes in fine-scale timing of daily behavioral rhythms compared to controls. We also found changes in the molecular circadian core clockwork in response to both size selective mortality treatments. These changes disappeared in the clock output pathway because both size-selected lines showed similar transcription profiles suggesting the presence of a molecular switch.

Main conclusion

Our experimental harvest left an asymmetrical evolutionary legacy in group risk-taking behavior and in fine-scale daily behavioral rhythms. Yet, the overall timing of activity showed evolutionary resistance probably maintained by a molecular switch. Our experimental findings suggest that human-induced changes in size selective mortality can have consequences for behavior and physiological processes in marine ecosystems.

Thematic area

Adaptation, Populations and species

Vasal budding, a new form of non-embryonic development in the colonial ascidian *Polyandrocarpa zorritensis*

Marta Scelzo¹, Marie Deschler^{1,2}, Alexandre Alié¹, Stefano Tiozzo¹

¹ CNRS, Laboratoire de Biologie du Développement de Villefranche-sur-Mer (LBDV), Sorbonne Université (France)

² CNRS, Laboratoire de Biologie des organismes et écosystèmes aquatiques (BOREA), Université du Havre (France)

Aim

Tunicates are the closest relatives of vertebrates able to regenerate a new adult body from adult epithelia and/or mesenchymal cells via asexual budding and whole-body regeneration, two forms of non-embryonic development (NED). Interestingly, this regenerative capacity is scattered across the whole sub-phylum, with species able to undergo NED (colonial) closely related to species where this regenerative capabilities are absent or reduced (solitary). This suggests that NED has been acquired or lost several times among the group. A recent phylogeny of the Styelidae family suggests that the colonial species *Polyandrocarpa zorritensis* have acquired the capability of NED independently from other budding species. However, a detailed description of the cells and tissues responsible for NED in this species was missing.

Methods

Histological and ultrastructural analyses were carried in order to identify the stages of NED under laboratory conditions and characterize the nature of the tissues and cells involved. The participation of cell proliferation has been also tested by EdU assays and anti-PHH3 immunostaining.

Results

In *P. zorritensis*, new buds develop through an invagination of vascular epidermis with contribution of mesenchymal cells. This type of NED was undescribed before and we called it “vasal budding”. During the early stages of vasal budding, undifferentiated mesenchymal cells named hemoblasts cluster and proliferate at the budding point. Hemoblasts are known as putative stem cells responsible for NED in other colonial ascidian and preliminary data on *P. zorritensis* suggest that hemoblasts express the stem cell marker Integrin- α -6 at the onset of organogenesis during vasal budding.

Main conclusions

Overall this work generates basic biological understandings of a new organism, *Polyandrocarpa zorritensis*, suitable for further comparative studies on the cellular and molecular mechanisms of NED in tunicates and their evolution.

Thematic areas

Biodiversity, Macroevolution

Complex evolutionary history obscures perception of species identity within nudibranch genus *Zelentia* (Gastropoda: Fionidae)

Dimitry M. Schepetov^{1,2}, *Valentina G. Tambovtseva*², *Tatiana I. Antokhina*³, *Irina A. Ekimova*¹

¹ Department of Invertebrate zoology, Lomonosov Moscow State University, Moscow, Russia

² Laboratory of evolutionary developmental biology, N.K. Koltzov Institute of Developmental Biology RAS, Moscow, Russia

³ Laboratory of Marine Invertebrate Morphology and Ecology, A.N. Severtsov Institute of Ecology and Evolution RAS, Moscow, Russia

Aim

Taxonomic studies of marine biota can give uncertain results when they are based on sparse morphological differences and molecular delimitation alone without considering biogeographical patterns of the studied region. Recent revisions of the nudibranch family Fionidae *s.l.* (Gastropoda: Nudibranchia) led to the erection of a number of new taxa on species, generic and family levels. However, in several cases the boundaries between these taxa remain unclear. We aimed to update molecular and morphological data on *Zelentia pustulata* species complex in Atlantic and Arctic to clarify its identity and provide a new prospect for further revision of this group.

Methods

The molecular study included five markers (COI, 16S, H3, ITS1 and ITS2) and implemented Bayesian and Maximum likelihood phylogenetic approaches. Species delimitation and intraspecific divergence were investigated with ABGD, PTP and GMYC methods, and TCS-based haplotype analysis. Ancestral area reconstruction and divergence time estimation were implemented to uncover evolutionary history of this species. Morphological analysis included standard anatomical techniques and SEM.

Results

Our integrative analysis and phylogeographic approach showed that *Zelentia pustulata* and *Z. roginsskiae* represent a single species, which is highly heterogeneous at both morphological and molecular levels. Divergence times estimation suggests a complex evolutionary history for this species, following Pleistocene glacial conditions in Arctic and Atlantic region that is reflected by the mtDNA divergence.

Main conclusion

We propose to synonymize *Zelentia pustulata* and *Z. roginsskiae*. Forthcoming Arctic biodiversity studies should consider the necessity to account for complex evolutionary history and phylogeographical patterns in delimitation of taxa inhabiting regions undergoing rapid environmental changes.

Thematic Areas

Populations and Species, Biodiversity

Introgression in the genus *Chionodraco*: a population genomic approach

*Luca Schiavon*¹, *Santiago Ceballos*^{2,3}, *Michael Matschiner*^{4,5}, *Emiliano Trucchi*⁶, *Mario La Mesa*⁷,
*Lorenzo Zane*¹, *Chiara Papetti*¹

¹Biology Department, University of Padova, Padova, Italy

²Instituto de Ciencias Polares, Ambiente y Recursos Naturales (ICPA), Universidad Nacional de Tierra del Fuego (UNTDF), Ushuaia, Argentina

³Centro Austral de Investigaciones Científicas (CADIC-CONICET), Ushuaia, Tierra del Fuego, Argentina

⁴Zoological Institute, University of Basel, Basel, Switzerland

⁵Department of Biosciences, Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo, Oslo, Norway

⁶Department of Life and Environmental Sciences, Marche Polytechnic University, Ancona, Italy

⁷ISMAR-Istituto di Scienze Marine - UOS Ancona, Italy

Aim

Hybridization is increasingly recognized as a widespread phenomenon which can impact the evolutionary history of the species in different ways. Previous studies indicated the presence of hybridization and introgression inside the genus *Chionodraco*, a group of three Antarctic fish (order Perciformes, suborder Notothenioidei, family Channichthyidae). Notothenioid fishes dominate the cold waters around the Antarctic continent in terms of species diversity and biomass and represent one of the most important examples of adaptive radiation in the marine realm. Our aim is to better understand the pattern of introgression and population structure inside the *Chionodraco* genus taking advantage of genomic data.

Methods

By means of single-RAD-sequencing protocol, we genotyped 65 individuals belonging to the genus *Chionodraco*, plus 20 individuals of *Chaenodraco wilsoni*, the sister species of the *Chionodraco* genus, as an outgroup. The population structure and the occurrence of introgression among the target species were investigated by nearest neighbour haplotype coancestry and D-statistics analyses.

Results

We detected a high level of incomplete lineage sorting among the three *Chionodraco* species and provided previously undetected evidence of ancient hybridization between *C. hamatus* and *C. myersi*. For *C. rastrispinosus*, our approach identified a very small population differentiation among the Antarctic Peninsula, South Orkney Islands and the recently found population in the Weddell Sea.

Main conclusions

Our study indicates that the evolutionary history of the Antarctic ichthyofauna is more complex than previously known. Genomic data can shed light on the dynamics of present and past population structure of Antarctic fishes and contribute to the description and monitoring of the biodiversity of this unique group of fish.

Thematic area

Populations and species

Resolving Animal Photopigment Relationships Reveals the Artefactual Nature of Enigmatic Opsin Families

Benjamin Schlossman¹, Davide Pisani¹, Roberto Feuda²

¹ University of Bristol, United Kingdom

² University of Leicester, United Kingdom

Aim

Opsins are light sensitive photopigment proteins responsible for phototransduction, the conversion of light to an electrical potential. Opsins are present in every animal except sea sponges and are crucial in the role of animal vision. Early opsin evolution, focusing on marine life, is critical to our understanding of how nervous systems evolved in the early animals, although no agreement has been reached on the relationships between opsin subfamilies.

Methods

We resolved opsin family relationships with the reanalysis of a previous dataset that proposed several novel yet enigmatic clades; the relationships between novel clades and the major opsin families is contentious. We also construct a new, high quality dataset that reveals a simple structure of opsin classification, with particular focus on early marine Phyla: Cnidaria and Ctenophora.

Results

We show that the use of destabilising outgroups and inadequate models of substitution play a role in the erroneous proposal of additional opsin paralogs in the Bilateria.

Main conclusion

The new dataset improves the taxonomic sampling of non-bilaterian, marine animals, providing evidence for the diversification of all opsin subfamilies in the eumetazoan and a single origin for the evolution of nervous systems in Metazoa.

Thematic area

Macroevolution

Application of DNA metabarcoding in a high-frequency zooplankton biodiversity monitoring project

Schroeder A.^{1,2}, Stanković D.³, Pansera M.¹, Pallavicini A.², Camatti E.¹

¹ National Research Council (CNR ISMAR) Venice, Italy

² University of Trieste, Faculty of Environmental Life Sciences, Trieste, Italy

³ Marine Biology Station Piran, National Institute of Biology, Slovenia

Aim

Monitoring zooplankton biodiversity is crucial when studying marine ecosystem processes as well as a support for ecosystem-based management efforts. However, as the morphological assessments of zooplankton biodiversity are labour intensive, and therefore the characterization of its spatio-temporal variability would often require an unsustainable effort, there is a rising need for fast and more cost-effective methods. After having successfully evaluated the suitability of DNA metabarcoding for zooplankton biodiversity assessments by comparing it to the morphological identification, this study aims to apply this molecular method on a monitoring project with high spatial and temporal frequency in the Venice Lagoon (Italy).

Methods

In 16 stations within the Venice Lagoon zooplankton samples were monthly collected with a 200 µm standard net from April 2018 to March 2019 together with environmental parameters. From the resulting 192 samples, the DNA was extracted, a fragment of the COI gene was amplified, and the sequencing was performed with the Ion Torrent PGM System. The bioinformatic pipeline included quality filtering, sequence corrections as well as different similarity thresholds to recover possibly all putative metazoan sequences.

Results

The analysis resulted in over ten million raw sequences and about 7 million sequences assigned more than 500 taxa belonging to 15 different phyla. The ecological analysis of the zooplankton community showed a differentiation both between different locations and months, following the seasonality of environmental changes throughout the year.

Main conclusion

The results highlight that DNA metabarcoding, with the advantage of increased sample processing speed and the broad taxonomic coverage, is a promising tool for surveying marine biodiversity, especially in large-scale monitoring programs and biodiversity assessments of ecosystems with high spatial and temporal variability.

Thematic Area

Biodiversity

Benthic microbial community structure across the Adriatic Sea: role of terrigenous inputs

*Matteo Selci**, *Federico Spagnoli*[°], *Rocco De Marco*[°], *Mario Tramontana*[§], *Elisa Dronighi*[§], *Enrico Dinelli*[¶], *Anna Annibaldi*[‡], *Elena Manini*[°], *Donato Giovannelli*^{*}

*University of Naples Federico II

[°]National Research Council of Italy

[§]University of Urbino "Carlo Bo"

[¶]University of Bologna

[‡]Polytechnic University of Marche

Aim

Prokaryotes play a key role in the coastal environment, regulating numerous biogeochemical processes relevant to ecosystem health. Along coastal regions, flowing rivers represent the first vector of transport of sediments from the land. With them, huge quantities of substances like nutrients, organic matter, and chemical compounds are transferred to the marine ecosystem. Transported nutrients and compounds spread along the marine coastal regions, where under different depositional systems they interact with benthic microbial communities determining their ultimate fate.

Methods

To assess the influence of different depositional environments and trophic sources on prokaryotes diversity and distribution, we investigated the variability of benthic prokaryotic communities in the surface sediments of 80 stations of the Middle and Southern Adriatic Sea, along the Italian coast.

Results

Investigated organic matter showed typical values of eutrophic environments. This was also confirmed from the prokaryotic diversity found, suggesting a strong role of the prokaryotes in organic matter cycling. To understand which factors affected the prokaryotic community structure, the large-scale microbial diversity dataset was correlated to different environmental data.

Since there was a lack of linear relationships between variables, different models were used.

A non-linear combination of sediments geochemistry, grain size distribution, and station-coastline distance was identified through General Additive Models analysis as the major factors explaining the variation in the prokaryotic distribution. These variables were associated with Adriatic sediment deposits coming from Alpine and Apennines river inputs, which suggest a strong influence in controlling the characteristics of the sediments, and the distribution of trophic resources and prokaryotic abundances.

Main conclusion

These observations suggest a strong role of the sedimentary depositional regime in controlling the benthic microbial community structure, potentially influencing the functions performed at the sediment-water interface for the flux of nutrients to the entire benthic community.

Thematic Areas

Populations and Species, Biodiversity

Fishing for the Evolutionary Origins of the Endocannabinoid System

*Rosa Maria Sepe**, *Luigi Caputi**, *Salvatore D'Aniello**, *Paolo Sordino**

*Department of Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn – Naples – Italy

Aim

The endocannabinoid system is a widespread neuromodulatory system, comprised of cannabinoid receptors, endogenous cannabinoids, and the enzymes responsible for the synthesis and degradation of the endocannabinoids. It is capable of regulating neuronal excitability and it is involved in a variety of physiological processes that regulate the adaptation to environmental stimuli. Our aim is to study the evolution of cannabinoid signaling and assess the phylogenetic distribution and evolutionary origins of this ancestral pathway.

Methods

To investigate the phylogenetic distribution of proteins involved in the endocannabinoid system, we submitted human proteins as query sequences in BLAST server. The homologous sequences, found in a wide range of animal species, were selected and aligned to reconstruct maximum-parsimony phylogenetic trees, using the SeaView software.

Results

The cannabinoid receptors, as mediators of cannabinoid physiological actions, are the focal points for a phylogenetic survey of cannabinoid signaling. They are unique to the phylum Chordata and, as such, they have a rather restricted phylogenetic distribution in the animal kingdom. However, other components of the cannabinoid system are evolutionary more ancient than the cannabinoid receptors. Orthologues of endocannabinoids synthesis and degradation enzymes and of cannabinoid receptor interacting protein are found in a wide range of animal species, such as deuterostomian and protostomian invertebrates and basal invertebrates.

Main conclusion

The presence of endocannabinoid signaling pathways in non-mammalian vertebrates and invertebrates can be of intrinsic scientific interest because it identifies a widespread phylogenetic distribution of this intercellular messenger system, which can be considered to be evolutionarily ancient and therefore of fundamental importance in the diverse branches of the tree of life.

Thematic Areas

Macroevolution, Adaptation

Molecular Evolution of Olfactory Receptors through Actinopterygii lineage

Liliana Silva^{1,2}, Tito Mendes^{1,2}, Agostinho Antunes^{1,2,}*

¹ CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research,

² Department of Biology, Faculty of Sciences, University of Porto

Aim

Recognition of odors is extremely important for intra/interspecific communication and species survival. Olfactory receptors (ORs) perform a major role in detecting odors. The several ORs classification systems available are full of ambiguities mainly related with existence of a gene repertoire highly diverse across vertebrate species (the presence of a single intact gene in elephant sharks compared to the more than 1,000 genes in some mammalian species). In Actinopterygii fish, the OR family was characterized in some punctual species but little is known about the gene repertoire and molecular evolution in most fish. Thus, the aim was to explore the diversity of OR genes among fish lineages.

Methods

For that purpose, 12 Actinopterygii species genomes, from basal to apical lineages, were considered. A gene extraction protocol was applied followed by ML phylogenetic reconstructions. Then, adaptive selection analyses were conducted at the codon level targeting to assess the ω ratio and unravel the evolutionary patterns of fish ORs that can provide clues to support their proper classification.

Results

The analyses performed revealed that in Actinopterygii species OR genes varies between $50 < \text{ORs} > 300$ copies, with Teleostei species having a constraint in OR copy number when compared to ancestral fish lineages. The phylogenetic approach performed allowed to evaluate the old OR classification systems and to determinate, through an integrative view, 11 distinct OR families. Codon level analyses revealed that ORs families are mainly under strong negative selection.

Main conclusion

Actinopterygii fish present a diversified repertoire of OR genes with high duplication rates in ancestral fish lineages. The applied methodology helped to clarify the OR classification system. However, as future perspectives, further branch- and branch-site analyses must be performed in order to solve remaining questions and to clarify how selective pressures are acting on ORs evolution.

Thematic Areas

Adaptation, Biodiversity

Glimpsing the deep-sea *Chauliodus sloani* genome

Marco Sollitto¹, Rachele Cesaroni¹, Marco Gerdol¹, Alberto Pallavicini^{1,2}, Sergio Stefanni²

¹Department of Life Sciences, University of Trieste, Via Licio Giorgieri 5, 34127, Trieste, Italy; ²Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy

Aim

Chauliodus sloani (Bloch & Schneider, 1801) is an iconic deep-sea fish that was originally described from a specimen collected in the Western Mediterranean Sea (holotype: BMNH 1978.9.11.1). It is inhabiting meso- and bathypelagic zones of the Atlantic, Indian, and eastern Pacific Ocean. It belongs to the family of Stomiidae, and it shows several phenotypical adaptations to deep-sea life. Indeed, it presents an elongated and compressed body, two rows of photophores (bioluminescence-producing organs), hinged jaws, and well-developed teeth. Despite its newsworthy features, genomic resources on *C. sloani* are lacking, and there are only a few genomic data on deep-sea fishes. Therefore, in this work, we report a preliminary study on the genome assembly of *C. sloani*.

Methods

A large specimen was collected in the Strait of Messina. High-molecular-weight genomic DNA was extracted, and it was sequenced through Illumina paired-end sequencing strategy with a depth of 32X. A kmer-based analysis produced an estimated genome size of 1 Gb with a heterozygosity of 2.16%. These observations prompted us to implement long-read sequencing through PacBio platform. Approximately, 78 Gb of long reads were generated, and an ~80× coverage of sequence data was assembled adopting a hybrid assembly strategy.

Results

Raw long reads were corrected through FMLRC tool. Following this step, two different assemblers, HASLR and WTDBG2 were employed for a comparative analysis of the *de novo* genome assemblies. The tool *quickmerge* was used for improving assembly contiguity, merging the aforementioned assemblies. The completeness of the merged assembly, evaluated with the Benchmarking Universal Single-Copy Orthologs (BUSCO) software, reached 68.3%. Moreover, its contiguity (N50 = 320461bp) was definitively improved as confirmed by QUAST analysis.

Main conclusion

A high-quality genome assembly of *C. sloani* would pave the way towards comparative genomics studies, providing a first critical resource to investigate the key adaptation to deep-sea environment.

Thematic Areas

Populations and Species, Adaptation

Towards a curated sequence reference dataset for animal COI DNA metabarcoding

*Stanković D.*¹, *Schroeder A.*^{2,3}, *Greco S.*², *Carpona M.*², *Stefanni S.*⁴, *Pallavicini A.*²

¹Marine Biology Station Piran, National Institute of Biology, Slovenia

²University of Trieste, Faculty of Environmental Life Sciences, Trieste, Italy

³National Research Council (CNR ISMAR) Venice, Italy

⁴Stazione Zoologica Anton Dohrn, Naples, Italy

Aim

DNA metabarcoding combines DNA barcoding with high-throughput sequencing to identify different taxa within environmental communities. The COI is a standard universal barcode marker for animals, however a comprehensive, updated and accurate reference dataset of animal COI sequences has not been available so far. Here, we are presenting preliminary work on constructing such a reference. So far we have constructed a reference dataset of all Metazoa excluding Hexapoda.

Methods

We retrieved 1 004 333 sequences from NCBI, and extracted the COI sequence. We have quality checked the sequences by excluding those shorter than 300 bp, those with ambiguous nucleotides, human, bacterial and non-metazoan contaminations, and those sequences identified only above genus level. We clustered the remaining sequences at a 100% similarity and manually checked and resolved taxonomic discrepancies appearing above the family level, while we used the taxonomy of the last common ancestor for the discrepancies below this level.

Results

At the moment our reference dataset includes 445 731 COI sequences clustered at 100% similarity belonging to 114 614 taxa. Of these, 348 914 are singleton clusters, while 83 403, 11 740 and 1258 clusters share a species, genus or family taxonomy, respectively. We have tested the curated database on already published DNA metabarcoding projects targeting marine, freshwater and terrestrial ecosystems.

Main conclusion

A curated and updated reference database is a first step for the standardization of animal DNA metabarcoding studies enabling comparison of different DNA metabarcoding projects. In the future, we plan to expand the curated reference dataset to include Hexapoda sequences. Furthermore, we will cluster all the sequences in the dataset at 99% similarity to reduce redundancy and computational effort.

Thematic Area

Biodiversity

Field simulation of future marine heat waves on the seagrass *Posidonia oceanica*: the importance of plant origin to heat stress tolerance

*Patrizia Stipcich**, *Lazaro Marín-Guirao†*, *Paola Maglioli†*, *Arianna Pansini**, *Luigi Piazzì§*, *Federico Pinna**, *Gabriele Procaccini ¥*, *Giulia Ceccherelli§*

*Department of Architecture, Design and Urbanistic, University of Sassari, 07100, Italy

† Oceanographic Center of Murcia, Spanish Institute of Oceanography C/Varadero, 30740 San Pedro del Pinatar, Murcia, Spain

† Thermal power plant Fiume Santo - Fiume Santo S.p.A. - EP Produzione, Porto Torres, 07046, Italy

¥ Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

§Department of Chemistry and Pharmacy, University of Sassari, 07100, Italy

Aim

Marine Heat Waves (MHWs) have been increasing in intensity and frequency since the past century with impacts to marine ecosystems. Their effects on the seagrass *Posidonia oceanica* need to be deeply explored in order to predict its performance in the future scenario. Here the phenological change of the seagrass at MHWs of different intensity was estimated in a field experiment, testing if the origin of the plant may influence the resilience to the heat stress.

Methods

In order to simulate MHWs in the field, the Fiume Santo thermal power plant (North West Sardinia), which releases sea water up to 8°C warmer than the natural, was chosen. Because the released water remains stratified on the surface, specific structures to expose *P. oceanica* plants at 1 m deep were used. *P. oceanica* shoots taken from two donor sites (hot vs cold, North and West Sardinian coast, respectively) were transplanted in a common garden set up by three areas in front of the power plant, so that at each of them a different MHW intensity was simulated (high, medium and control temperature). After 8, 19 and 40 days of exposure some phenological variables were measured. The experiment was conducted in summer 2020.

Results

Overall, important effects of the three heat treatments on the transplanted plants were highlighted as there was a decrease in most of the variables considered. However, a different performance between plants collected in the two donor sites was found, with the former showing a higher resistance to the treatments.

Main conclusion

The impact on *P. oceanica* performance of the simulated MHWs in the field suggests that the future summer temperature due to climate change projection will threaten the plants, but that the impact will be context-dependent in relation to the pre-adaptation of the plants to the local environmental conditions.

Thematic Areas

Populations and Species; Adaptation

Parental phenotype and exposure to ocean acidification conditions affects the molecular responses of gills in the offspring of a coral reef fish

Sneha Suresh¹, Megan J Welch², Philip L Munday², Timothy Ravasi³, Celia Schunter¹

¹Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong SAR

²Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Australia

³Marine Climate Change Unit, Okinawa Institute of Science & Technology Graduate University, Onna-son, Okinawa, Japan

Aim

Ocean acidification can have wide-ranging impacts on marine ecosystems. While short-term studies have reported negative effects of future predicted CO₂ levels on a variety of marine organisms, some populations might have the potential to acclimate and/or adapt to elevated CO₂ levels across generations. Understanding the capacity of marine organisms to adapt to future CO₂ levels will enable us to better predict the biological impacts of ocean acidification. This study therefore focuses on the effects of 1) variation in parental tolerance and 2) parental exposure to elevated CO₂, on the molecular responses in the gills of the offspring to elevated CO₂ in a coral reef fish.

Methods

Adult *Acanthochromis polyacanthus* were collected from the Great Barrier Reef and classified as either having sensitive or tolerant behavioural phenotype based on their response to chemical alarm cues after elevated CO₂ exposure. Breeding pairs of each phenotype were kept in control or elevated CO₂ conditions with the resulting offspring reared either in control and elevated CO₂ conditions. RNA was extracted from the gills of the offspring, sequenced, and analyzed.

Results

Cross-generational exposure to elevated CO₂ had a significant impact on gene expression patterns of the offspring. Offspring from parents exposed to elevated CO₂ exhibited approximately half the differentially expressed genes compared with offspring in the within-generation elevated CO₂ treatment. The parental phenotype also influenced gene expression, with 732 and 183 genes differentially expressed in the control and within-generation CO₂ treatment respectively, when comparing directly between offspring of the two parental phenotypes.

Main conclusion

Fish whose parents were exposed to elevated CO₂ had an enrichment of genes involved in ion regulation and homeostasis related functions and decreased expression of stress response genes compared to the within-generation treatment.

Thematic areas

Adaptation, Population and Species

Connectivity patterns among seagrass populations of the western coast of Africa inferred from genetic markers and oceanographic currents suggest a role of grazers in seed dispersal

Ana Tavares¹, Jorge Assis¹, Rita Patrício², Gareth Pearson¹, Ester Serrão¹

¹ CCMAR - Centre of Marine Sciences, University of Algarve, Faro, Portugal

² MARE – Marine and Environmental Sciences Centre, ISPA – Instituto Universitário, Lisbon, Portugal

Aim

Movement is a vital evolutionary and ecological action for life that affects genetic diversity, persistence and connectivity of populations. Species that occupy similar ecological niches, such as seagrasses, can reveal different dispersal extent in the marine environment. In this study we use the seagrass species *Halodule wrightii* Ascherson, 1868, due to its restricted tendency for self-dispersal, to estimate the genetic connectivity along its eastern Atlantic distributional range, where few known occurrence reports are known, to assess the influence of abiotic (ocean currents) versus biotic (grazers) vectors in the observed genetic patterns.

Methods

To test the hypothesis, eight genetic markers (microsatellites) were used to genotype *Halodule wrightii* samples from eleven localities, from Mauritania to Angola including Cape Verde and São Tomé and Príncipe islands. Population genetic structure was analyzed with Bayesian assignment methods and factorial correspondence analysis. Biophysical modelling methods were used to infer the influence of oceanographic currents on *Halodule wrightii* populations genetic structure.

Results

Halodule wrightii populations revealed low genetic diversity, a considerable variation of genotypic diversity and a differentiation between north and south populations of the equator. The biophysical models of simulated transportation of seagrass fragments mediated by ocean currents, revealed a very low probability of connectivity between populations along the target area, suggesting that biotic transportation might have an important role in this species dispersal.

Main conclusion

Our findings provide the first insight into seagrass metapopulation connectivity along the tropical west Africa coast, but future studies are essential to understand the true animal dispersal potential in this region.

Thematic areas

Biodiversity, Populations and Species

Growth rate of *Hermodice carunculata* (Annelida, Amphinomidae) under laboratory conditions

Andrea Toso*, Marta Mammone*, Adriana Giangrande*, Stefano Piraino*

* Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce.

Aim

Hermodice carunculata (Pallas, 1766) is a large-sized polychaete with an opportunistic scavenger feeding behaviour. Due to its northward spread along the Italian coasts, it is considered as a biological indicator of the tropicalization of the Mediterranean Sea. To understand its population dynamics and local invasiveness potential, laboratory growth rate estimations were carried out starting from small specimens sampled at shallow waters off Santa Caterina (Ionian Sea, Apulia, Italy).

Methods

Six small specimens of *Hermodice carunculata* (mean size $11,4 \pm 1,9$ cm length; $3,4 \pm 1,6$ g weight; mean age 13-15 months) were transferred to a 100 l aquarium at $T = 22$ °C, salinity 35-38‰, with a constant LD 12-12 photoperiod, and fed *ad libitum* twice a week with a diversified diet. Specimens with different color patterns were chosen for a better identification over the experiment. Individual weight, length, and number of chaetigers were monthly assessed from October 2019 to July 2020. At the end of the experiment, a classical hematoxylin-eosin histological analysis was carried out for determining the state of sexual maturity of the cultured specimens.

Results

Over the course of the 10-months experiment, the mean size of cultured fireworms increased from $11,4 \pm 1,9$ cm to $22,4 \pm 3,5$ cm in length, and from $3,4 \pm 1,6$ g to $17,5 \pm 3,5$ g in weight, with a daily growth rate of $0,054 \pm 0,008$ g/day. At the end of the experiment coelomic cavities were filled by sperms or oocytes at different state of maturations. The distinctive colour pattern of each specimen was maintained over the course of the experiment.

Main conclusion

Under laboratory conditions, fireworms exhibited fast growth rate with large dietary adaptability to different food items, and reached sexual maturity during their second year of life. The occurrence of large specimens in wild populations may be indicative of iteroparous reproduction. These features may be key to understand the high invasive potential of this species across the Mediterranean basin.

Thematic Areas

Populations and Species, Biodiversity

The non-vertebrate chordate *Ciona robusta* reveals novel insights in Onecut-mediated photoreceptor differentiation

Quirino Attilio Vassalli^{1§}, Chiara Colantuono², Federica Salatiello¹, Maria Luisa Chiusano², Paolo Sordino¹, Annamaria Locascio¹

¹ Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms, 80121 Napoli, Italy.

² Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.

§ Present address: Scripps Research 10466 N Torrey Pines Rd, La Jolla, CA 92037, USA.

Abstract

Photoreceptors are neurons highly specialized for sensing light stimuli and have considerably diversified during evolution. The genetic mechanisms that underlie photoreceptor differentiation and accompanied the progressive increase in their complexity and diversification are a matter of great interest in the field. By using transgenic and reverse genetics approaches on the *Ciona robusta* model system, we aimed to identity the Oc downstream-acting factors that mediate specific tasks in the differentiation of photoreceptor cells. In this study, we contribute in understanding the cascade of events that direct the formation of the increasingly complex eye structures of chordates.

Methods

We used transgenic perturbation of the *Ciona* Oc protein to show its requirement for ciliary photoreceptor cell differentiation. Then, we performed a RNAseq analysis between the trans-activation and trans-repression Oc phenotypes and an *in silico* comparison of the *C. robusta* and mouse mutant RNA-Seq datasets.

Results

The Oc transcription factor emerges as a key element for *Ciona* photoreceptors differentiation. Transcriptome profiling between the trans-activation and trans-repression Oc phenotypes identified differentially expressed genes that are enriched in exocytosis, calcium homeostasis and neurotransmission. In particular, its downstream genetic pathway evidenced core components of the molecular machinery for neurotransmission of retinal cell types in chordates. Finally, comparison of RNA-Seq datasets in *C. robusta* and mouse identified a set of Oc downstream genes conserved between tunicates and vertebrates.

Main conclusions

- The transcription factor Oc is necessary for ciliary photoreceptor cells differentiation in ascidians.
- Comparative transcriptomics of Oc perturbation in ascidian and mouse highlights the existence of effector genes involved in neurotransmission of retinal cell types.
- Full comprehension of Oc role in photoreceptor differentiation could represent an important turning point to understand the process that contributed to cone and rod diversification in vertebrates.

Thematic Areas

Macroevolution, Populations and Species

Mixing genomes separated 20 million years ago: whole genome sequencing to understand the hybridization process between sea turtle species

Sibelle T. Vilaça¹, Omar Rota-Stabelli², Riccardo Piccinno², Luciano Soares³, Alan Bolten, Karen Bjorndal³, Silvia Fuselli¹, Giorgio Bertorelle¹.

¹ Department of Life Sciences and Biotechnology, University of Ferrara.

² Department of Sustainable Agro-ecosystems and Bioresources, Fondazione Edmund Mach.

³ Archie Carr Center for Sea Turtle Research, University of Florida.

Aim

Sea turtles comprise seven extant species, and six are classified as endangered. These species have diverged for at least 20 million years, and despite their long divergence time, four species are known to hybridize on the Brazilian coast. In the largest nesting population in Brazil, 30-40% of nesting females morphologically identified as hawksbills (*Eretmochelys imbricata*) are genetically identified F1 hybrids between loggerheads (*Caretta caretta*) and hawksbills. First generation hybrids between loggerheads and olive ridleys (*Lepidochelys olivacea*) are also found, although not as frequently. Hybridization is possibly driven by the population decline that reduces the chance to find a mate of the right species. Hybrids can backcross with both parental species and produce viable offspring. Previous research has shown that loggerhead x hawksbill hybrids and pure species have similar reproductive output, hybrids are noticeably bigger than pure species, and the hybrid offspring have the same fitness as pure offspring from both species.

Methods

To study and understand species hybridization under the extreme conditions of a secondary contact between highly divergent genomes, we are using whole genome sequences from five sea turtle species including population data for loggerheads, hawksbills, and their F1 hybrids.

Results

We will present preliminary results on population dynamics reconstructed from genomic data, which can be useful to understand the impact of population size changes on the hybridization process. Phylogenies from five species show that chromosomes have unique evolutionary rates and different putative signs of hybridization. We will also present a general description of the genetic diversity of all species and putative genomic regions associated with ancient hybridization events. Our goal is to understand the consequences of hybridization for the conservation of the Brazilian sea turtle population.

Thematic Areas

Biodiversity, Populations and Species



POSTERS

Genetic structuring in endangered yellow-nosed albatross species complex.

Dilini Abeyrama, Zach Dempsey, Peter Ryan and Theresa Burg

Naso-oral region analysis reveals a phylogenetic pressure in the Houndshark.

Stefano Aicardi, Alessio Longo, Lorenzo Gallus and Sara Ferrando

Multidrug-resistant marine bacteria isolated from the shallow hydrothermal vent systems of Panarea (Aeolian Islands).

Gabriele Andreatta, Caroline Broyart, Charline Borghgraef, Karim Vadiwala, Vitaly Kozin, Alessandra Polo, Andrea Bileck, Isabel Beets, Liliane Schoofs, Christopher Gerner and Florian Raible

Potential crypticity within two decapod (Crustacea) genera: *Galathea* (Fabricius, 1792) and *Eualus* (Thallwitz, 1891).

Erika Arcadi, Monique Mancuso, Marilena Sanfilippo, Danilo Malara, Franco Andoloro, Valentina Esposito, Cinzia De Vittor and Teresa Romeo

A comparison of metabolic and behavioural responses in sea urchins from different environments during long-term exposure to seawater acidification.

Davide Asnicar, Marco Munari and Maria Gabriella Marin

Morphology and reproduction in the *Hapalocarcinus marsupialis* Stimpson, 1859 species complex (Decapoda: Brachyura: Cryptochiridae).

Susanne Bähr, Sancia E.T. van der Meij and Magnus Johnson

Negotiations Over Parental Care: A Test of Alternative Hypotheses in the Clown Anemonefish.

Tina Barbasch, Rebecca Branconi, Robin Francis, Madison Pacaro, Maya Srinivasan, Geoffrey Jones and Peter Buston

An endocrine signaling integrates energy homeostasis and lunar phase to regulate aspects of growth and sexual maturation in *Platynereis*.

Anna Benvenuto, Andrea Bellodi, Riccardo Melis, Antonello Mulas, Alice Ferrari, Rita Cannas, Alessia Cariani and Maria Cristina Follesa

Digging into bivalve miRNAomes: between conservation and innovation.

Enrico Bortoletto, Umberto Rosani, Paola Venier and Bastian Fromm

Ecological and social constraints combine to promote evolution of non-breeding strategies in clownfish.

Rebecca Branconi, Tina Barbasch, Robin Francis, Maya Srinivasan, Geoffrey Jones and Peter Buston

Nitric Oxide and Retinoic Acid pathways crosstalk during amphioxus development.

Filomena Caccavale, Annona Giovanni, Lucie Subirana, Hector Escriva, Stephanie Bertrand and Salvatore D'Aniello

Evolution of the TRH neuropeptide pathway and its function in the regulation of echinoderm larval growth.

Maria Cocurullo, Natalie Wood, Paola Oliveri and Maria Ina Arnon

Perceived complexity and distinct boundaries: a revision of calcareous sponge *Leucosolenia complicata* in the White Sea.

Sheena Conforti and Federica Costantini

Comparative analysis of gene networks in marine and terrestrial angiosperms.

Emanuela Dattolo, Luca Ambrosino, Miriam Ruocco, Gabriele Procaccini and Maria Luisa Chiusano

Molecular and morphological identification of *Pseudodiaptomus marinus* strains from Italian waters.

Iole Di Capua, Rosa D'Angiolo, Elisa Camatti, Ylenia Carotenuto, Alenka Goruppi, Alessandra de Olazabal, Marco Pansera, Isabella Percopo, Valentina Tirelli, Giacomo Zagami and Marco Uttieri

Stress granules in *Ciona robusta*: molecular evolution of TIA-1-related nucleolysin and tristetraprolin and gene expression studies under metal-induced stress conditions.

Laura Drago, Lorian Ballarin and Gianfranco Santovito

Speciation drivers in high latitudes: systematics and phylogeography of the nudibranch family Coryphellidae (Gastropoda: Nudibranchia).

Irina Ekimova, Alexander Ereskovsky, Dimitry Schepetov, Daria Tokina, Alexandra Koinova and Andrey Lavrov

The Pandora's Box: morphological and genetic diversity within the genus *Amphiglena* Claparède, 1864 (Sabellidae, Annelida) in the Mediterranean Sea.

Irina Ekimova, Ángel Valdés, Manuel Malaquias, Cessa Rauch, Anton Chichvarkhin, Anna Mikhлина, Tatiana Antokhina and Dimitry Schepetov

Identification of microRNAs and piRNAs involved in the sea urchin *Paracentrotus lividus* germ line specification.

Ines Fournon Berodia, Francesca Rizzo, Konstantinos Geles, Danila Voronov and Maria Ina Arnone

The Internal Transcribed Spacer 2 (ITS2) as a new barcoding marker in nudibranchs (Gastropoda: Heterobranchia): an approach using sequence and secondary structure information.

Matteo Garzia, Paolo Mariottini, Daniele Salvi and Giulia Furfaro

Strong macrobenthic community differentiation among sub-Arctic deep fjords on small spatial scales.

Adriana Giangrande, Matteo Putignano, Margherita Licciano and Maria Cristina Gambi

Intelligent networks: new perspectives in the application of Internet of Things for marine investigations.

Francesca Glaviano, Valerio Zupo, Mirko Mutalipassi, Thomas Viel, Maria Cristina Buia and Maria Costantini

Transcriptomic adaptations of Cryonotothenioidea to the antarctic environment and response to heat stress.

Samuele Greco, Anastasia S. Gaetano, Federico Ansaloni, Gianfranco Santovito, Giuseppe Scapigliati, Alberto Pallavicini, Piero G. Giulianini, Marco Gerdol and Andrea Miccoli

A pre-screening of the presence of antibiotics in traces in two large pelagic fish in the strait of Messina.

Valentin Kokarev and Henning Reiss

The DNA-Methylation machinery of the mollusc *M. galloprovincialis* and its essential role in development.

Claudia La Vecchia, Marco Gerdol, Maria Strazzullo, Pasquale De Luca, Stefania Gorbi, Alberto Pallavicini, Francesco Regoli and Enrico D'Aniello

***Ciona robusta* reveals specific gut immune adaptations to a microbial world.**

Assunta Liberti, Paolo Sordino and Larry J. Dishaw

Sperm competition intensity drives ejaculate performance mediated by seminal fluid in the black goby *Gobius niger*.

Lisa Locatello, Oliviero Borgheresi, Andrea Pilastro and Maria B. Rasotto

Genetic variation analysis provides insights into diatom population genomics.

Svenja Mager, Francesco Manfellotto, Maria Valeria Ruggiero, Viviana Di Tuccio, Monia Russo, Campbell Lisa, Marina Montresor, Remo Sanges and Mariella Ferrante

Molecular Evolution of Olfactory Receptors through Actinopterygii lineage.

Monique Mancuso, Erika Arcadi, Danilo Malara, Marilena Sanfilippo, Pietro Battaglia, Franco Andaloro, Nunziacarla Spanò and Teresa Romeo

Evolution of the AP-1 transcriptional complex: identification of novel components in the ascidia *Ciona robusta*.

Pina Marotta, Federica Salatiello, Luca Ambrosino, Federica Berruto, Maria Luisa Chiusano and Annamaria Locascio

Symbiont recognition and establishment in the nudibranch *Berghia stephanieae*.

Jenny Melo Clavijo, Sabrina Bleidißel, Angelika Preisfeld and Gregor Christa

***Cassiopea* Ephyra Development Across Separate Feeding Regimes.** Kaden Muffett

Different fates of seagrasses in an era of ocean warming.

Manh Hung Nguyen, Peter Ralph, Lázaro Marín-Guirao, Mathieu Pernice and Gabriele Procaccini

Transcriptional responses of *Posidonia oceanica* under multiple stresses: the influence of the native environment.

Jessica Pazzaglia, Alex Santillán-Sarmiento, Miriam Ruocco, Emanuela Dattolo, Antonio Terlizzi, Lázaro Marín-Guirao and Gabriele Procaccini

Natural selection in the evolutionary divergence between two penguin species.

Federica Pirri, Lino Ometto, Silvia Fuselli, Lorenzo Zane and Emiliano Trucchi

Exploring variations in personality and morphometric traits in wild juveniles of sand smelt captured in different environments and with different fishing techniques in the Venetian Lagoon.
Federica Poli, Giovanni Polverino, Pietro Antolini, Alberto Nóvoa Abelleira and Carlotta Mazzoldi

Is it possible to predict the distribution of a rare parasitic species? The challenging case of *Savalia savaglia* (Bertoloni, 1819) (Cnidaria, Anthozoa).
orcuato Pulido Mantas, Cecilia Varotti and Carlo Cerrano

Mass mortality of *Pinna nobilis* in Sardinia: phylogenetic inference on the etiological agents.
Daria Sanna, Fabio Scarpa, Ilenia Azzena, Piero Cossu and Marco Casu

Vasal budding, a new form of non-embryonic development in the colonial ascidian *Polyandrocarpa zorritensis*.
Marta Scelzo, Marie Deschler, Alexandre Alié and Stefano Tiozzo

Complex evolutionary history obscures perception of species identity within nudibranch genus *Zelentia* (Gastropoda: Fionidae).
Dimitry Schepetov, Valentina Tambovtseva, Tatiana Antokhina and Irina Ekimova

Introgression in the genus *Chionodraco*: a population genomic approach.
Luca Schiavon, Santiago Ceballos, Michael Matschiner, Emiliano Trucchi, Mario La Mesa, Lorenzo Zane and Chiara Papetti

Resolving Animal Photopigment Relationships Reveals the Artefactual Nature of Enigmatic Opsin Families.
Benjamin Schlossman, Davide Pisani and Roberto Feuda

***Centrophorus* spp. and the mystery of the deep-sea: how many species in the Mediterranean?**
Matteo Selci, Federico Spagnoli, Rocco De Marco, Mario Tramontana, Elisa Droghini, Enrico Dinelli, Anna Annibaldi, Elena Manini and Donato Giovannelli

Fishing for the Evolutionary Origins of the Endocannabinoid System.
Rosa Maria Sepe, Luigi Caputi, Salvatore D'Aniello and Paolo Sordino

Benthic microbial community structure across the Adriatic Sea: role of terrigenous inputs.
Liliana Silva

Glimpsing the deep-sea *Chauliodus sloani* genome.
Marco Sollitto, Rachele Cesaroni, Marco Gerdol, Alberto Pallavicini and Sergio Stefanni

Connectivity patterns among seagrass populations of the western coast of Africa inferred from genetic markers and oceanographic currents suggest a role of grazers in seed dispersal.
Ana Tavares, Jorge Assis, Rita Patrício, Gareth Pearson and Ester Serrão

Growth rate of *Hermodice carunculata* (Annelida, Amphinomidae) under laboratory conditions.
Andrea Toso, Adriana Giangrande and Stefano Piraino