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ABSTRACT BOOK

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Understanding heat stress resistance in *Orbicella faveolata* from the Florida Keys using gene networks analyses

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Florida Keys coral reefs have experienced seven mass bleaching events since 1987. Many Keys reefs have lost much of their coral cover, however, some inshore reefs can reach >40% of coral cover. It has been hypothesized that the persistence of higher inshore coral cover may indicate the corals at these sites are acclimatized and/or adapted to recurrent heat stress owing to naturally higher and more variable temperatures. To explore this hypothesis, we subjected 3 inshore and 4 offshore genotypes of *Orbicella faveolata* to different temperatures (T = 30, 31, 32, 33ºC) for 35 days. The inshore genotypes were more heat resistant than those offshore. To elucidate the mechanisms behind this differential heat resistance, we assembled the *O. faveolata* transcriptome and used a systems genetics approach to show how certain gene 'modules' are related to specific genotypes, environmental variation, and to heat stress. We found several gene modules that were highly correlated to the genotype most resistant to heat stress. One particular module (M5) was correlated to higher levels of photosynthetic yield and was enriched with genes involved in 'translation', with a total of 63 small and large ribosomal subunit genes. There were also modules that correlated with photosynthetic yield independent of the genotypes, these were annotated with 'proteolysis involved in cellular protein catabolic process' that included the expression of 26 proteasome subunit genes. Overall these results show that ribosomal genes are playing an important role in the resilience of *O. faveolata* to high temperatures by increasing cellular translation activity.

The genome of *Lepidodermella squamata* (Dujardin 1841) and the evolution of developmental gene pathways in Spiralia

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Gastrotrichs are a group of aquatic microscopic animals inhabiting marine and freshwater interstitial environments and hold a key position for understanding the evolution of Spiralia. Despite their importance, gastrotrich transcriptomic resources are limited and draft genomes have not been reported. Here, we have produced a draft genome of the freshwater gastrotrich, *Lepidodermella squamata* (Dujardin 1841), using a metassembly approach involving the combination and reconciliation of multiple assemblies. Our genome assembly comprises 9,901 scaffolds with an N50 of 120,792 bp, representing the first comprehensive genomic resource for Gastrotricha. Genome analysis showed an SNP density of 1.2SNPs/100bp and a heterozygosity rate of 1.03%, while genome scanning for interspersed repeats and low complexity DNA sequences revealed that 18.48% of the *Lepidodermella* genome correspond to repeat elements. Most of the repeat landscape correspond to unclassified repeats (11.02%) following by long interspersed nucleotide elements (LINEs) (5.85%). Interestingly, short interspersed nuclear elements (SINEs) are absent in the *Lepidodermella* genome. By using transcript-based, homology-based, and ab initio gene prediction methods, we have annotated ~30 k gene models in the *Lepidodermella* genome. Ongoing comparative analysis of *Lepidodermella* protein coding genes with other gastrotrich transcriptomes and spiralian genomes suggests a dynamic evolution of developmental gene pathways in these taxonomic groups. Taken together, this work will provide insights into the evolution of gastrotrichs and spiralians, as well as a new genomic resource for a poorly investigated taxonomic group.

A multi-omic approach to reveal interactions between the hard clam and its pathogen QPX

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QPX is a protistan parasite that infects hard clams, *Mercenaria mercenaria*, often leading to the development of inflammatory masses (nodules) that result from prominent hemocyte infiltration to the infection site in an attempt to isolate and encapsulate parasite cells. Inside nodules, active host-pathogen interactions take place leading either to the death of the parasite or invasion of surrounding tissues and infection worsening. This presentation summarizes research aimed at unraveling host-parasite interactions using a complementary set of in vivo and in vitro approaches in conjunction with several omics methods. In vivo, we studied dual gene expression of QPX and clams to characterize molecular host-parasite interactions. This was done via RNA sequencing of nodule biopsies and contrasting these with RNA sequences generated from uninfected tissues from infected clams or from healthy clams, as well as genomic and transcriptomic information generated from cultured parasite cells. Analyses allowed the identification of QPX transcripts that are produced in clam tissues during infection. These included genes and molecular processes implicated in the secretory pathways of the parasite and secreted proteases suspected to play a primary role in QPX virulence towards clams. In parallel, we used proteomic methods to identify host plasma factors that recognize and bind parasite cells in vitro. These included prominent pattern recognition receptors (PRR) such as complement c1q-domain containing proteins and lectins. Results further showed that these PRR are induced upon infection. Altogether, these results provide valuable information on the molecular crosstalk between QPX and its clam host.

seqCAB (Sequence Conversion & Annotation with BLAST+): A BLAST utility to facilitate '-omics' analyses through multi-core parallelization and taxonomic annotation

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Rapid technological advancements in analytical biomolecular sequencing platforms have made '-omics' projects data-driven endeavors, generating a scramble for computationally robust open source bioinformatics tools. In particular, environmental metagenomic datasets present challenges for fast and accurate analyses of next generation sequencing (NGS) datasets. Bioinformatic pipelines for analyzing NGS datasets rely on BLAST+ (basic local alignment search tool) for querying large-scale reference databases (i.e., NCBI nucleotide and/or protein databases). BLAST uses the heuristic tradeoff between speed and sensitivity by optimizing specific levels of similarity between queries and biomolecular sequence databases. However, BLAST searches have become computationally intensive and time-consuming as query read length, sequence abundance and reference database sizes have dramatically increased. Here, we present the new BLAST+ utility, seqCAB (Sequence Conversion & Annotation with BLAST+), which improves the efficiency of the critical homology search through multi-core parallelization with message passing interface (MPI), harnessing the modularity of python libraries in four steps: 1. conversion (Biopython), 2. BLAST parallelization (MPI4py), 3. annotation reporting (Pandas and Multiprocessing), and 4. taxonomy assignment (Pandas and Multiprocessing). seqCAB was successfully executed on two different High Performance Computing platforms and Ubuntu using four datasets (i.e. three previously published protein datasets (76K, 240K and 1M sequences), and a metagenomics dataset (2M sequences) that was generated for this study using MinION third-generation sequencing technology. Our open source utility seqCAB reduces the time lag from sample collection to data analysis and reporting of findings, thus facilitating contemporaneous assessment of ecological systems through environmental metagenomics and metaproteomics.

Genomics of Adaptation

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Whole genome sequencing projects can have a major relevance in understanding health, genetic disease, species adaptive evolution and molecular diversification. Currently, multiple species are having their genomes completely sequenced, from simple organisms, such as bacteria (e.g. microbiomes), to more complex taxa, such as higher metazoans. This voluminous sequencing data generated across multiple organisms provides also the framework to better understand the genetic uniqueness of such species and related ones, allowing to explore the genetic changes underlining the evolution of diverse phenotypic and adaptive traits. Here, recent results from our group retrieved from comparative evolutionary genomic analyses of varied invertebrate species will be considered to exemplify the adaptive success of species into diverse environments and lifestyles. The findings pinpoint unique molecular products of critical relevance in species evolution and diversification, but also highlight genomic novelties of importance for environmental and biomedical research.

Functional and genomic consequences of mitonuclear coevolution in an intertidal copepod

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Allopatric populations of the intertidal copepod *Tigriopus californicus* show high genetic divergence and varying degrees of reproductive compatibility, with interpopulation hybrid offspring suffering from reduced fecundity, slower development, and mitochondrial dysfunction. This system is hence often used as a model for understanding the early stages of speciation. We assembled a chromosome-level reference genome for one population (~190 Mb across 12 scaffolds) and annotated ~15,500 genes. In addition of the reference population, we performed assembly of the genomes of seven allopatric populations, spanning the continuum of reproductive isolation. Consistent with previous reports, populations of *T. californicus* show extreme mitochondrial DNA (mtDNA) differentiation, with upwards of 23% divergence at the nucleotide sequence level. Across the nuclear genome, we detected elevated rates of protein sequence evolution in genes that are predicted to interact with proteins and RNAs encoded by the mitochondrial genome. These genes are involved in multiple cellular pathways essential for mitochondrial function, such as mtDNA transcription, translation, and oxidative phosphorylation. Our results are consistent with the hypothesis that rapid mitochondrial evolution imposes selection favoring compensatory substitutions on interacting nuclear counterparts within isolated populations, thereby providing a potential mechanism for causing hybrid breakdown of metabolic and life history performance and resulting in intrinsic reproductive isolation.

Whole-chromosome assembly and analysis of hybridogenetic lineages of the desert ant *Cataglyphis hispanica*

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Assembling complete chromosomes of large genomes is a technical challenge presenting a number of long-standing issues, such as the bridging of gaps that can be found in draft genomes or the presence of repeated sequences that conventional assemblers have trouble resolving. As such, many large arthropod genomes are in an unfinished state, comprising many more scaffolds than the expected number of chromosomes. Here, we present instaGRAAL, a fast, open-source program that uses chromosome conformation capture (Hi-C) data to scaffold contigs based on the collision frequencies between DNA sequences in the nucleus. instaGRAAL builds upon and improves our formerly published program GRAAL, which uses a simple polymer model to represent the expected spatial contacts between these sequences and an MCMC method to maximize the likelihood of this model (Marie-Nelly et al., 2014). We also perform a number of polishing procedures, pulling from long read data to further curate the assembly and validate it through a number of state-of-the-art metrics such as gene completeness. Our assembly software and polishing pipeline results in a pair of high-quality chromosome-level assemblies. When applied to the genomes of two hybridogenetic lineages of the desert ant Cataglyphis hispanica, instaGRAAL revealed large-scale structural differences that may account for their unusual reproductive strategies.

Similarities and differences between genomes of temperate and tropical corals

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Reef-building corals are threatened by local and global stressors. In the Caribbean, the sympatric corals *Acropora palmata* and *A. cervicornis* are restricted to a narrow thermal range and their populations have been declining over the last several decades as seawater temperatures continue to increase. While tropical corals exist within a narrow temperature range, the coral *Astrangia poculata* extends over much of the US Atlantic coast from the Gulf of Mexico to southern Massachusetts and survives temperatures of 0° – 30° C. We compared draft genome assemblies from these three coral species to describe genome architecture and identify a “core” coral genome. This approach elucidated species-specific and geographic variation in the coral genomes that may underlie temperature adaptation in these important ecosystem foundation species.

Copepod phylogenomics: orthology inference for target-capture marker development

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Copepods are an excellent system to study parasite evolution because this taxon contains a diversity for free-living and parasitic taxa, has multiple independent evolutions of parasitism, has colonized a wide variety of host taxa, and contains species with extremely divergent morphology. Questions regarding parasitism, like any other trait, are best addressed in the context of robust phylogenetic hypotheses, but a phylogeny for copepods is currently lacking – less than 3% of described species have been included in molecular phylogenetic studies. To build a better understanding of evolutionary relationships among the Copepoda, a taxon containing 12,000 described species, a highly scalable approach is needed to efficiently generate sequence data. This study has three goals: (1) to identify new molecular markers for copepod phylogenomics, and then to use these markers to (2) test the ordinal relationships of copepods recovered in prior phylogenetic studies, and (3) identify the sister taxon to copepods given that previous studies have conflicting results with generally low support. We analyzed only publicly available data consisting of 3 copepod genomes, 22 copepod transcriptomes, 3 outgroup (OG) genomes and 47 OG transcriptomes from other crustaceans using the tree-based orthology inference pipeline of Yang and Smith (2014). Few 1-to-1 orthologs across all taxa are identified, but we find over 2,000 orthologs among copepod (i.e., ingroup) taxa. The results of the orthology inference analyses are discussed, and we present phylogenies generated using ASTRAL and other methods. This approach is replicable in most taxa with available transcriptomic and/or genomic sequences.

BlobToolKit: Identification and analysis of non target data in all Eukaryote genome projects

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As we begin to sequence all of Earth's biodiversity our task becomes more challenging. Our targets are not only smaller but they are not from pristine lab environments. We cannot isolate the target from its environment, which will include the organism's microbiome, parasites, pathogens, host and food sources. As understanding of the importance of symbioses, especially bacterial-eukaryote symbioses, increases, it is also important to distinguish symbiont genomes from their hosts'. An assembly that erroneously mixes "cobiont" data with the target genome will be positively misleading, and when deposited in public databases such as the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) will contaminate the public record.

Contamination in assemblies can be identified through typical patterns of read coverage, GC content and taxonomic affiliation, an approach we have previously used in our BlobTools project. We are now developing BlobToolKit (http://blobtoolkit.genomehubs.org/), an interactive system for identification and classification of cobiont data in genome assemblies. BlobToolKit simplifies reproducible filtering of assemblies and raw data with a rich graphical interface, and publication-quality outputs.

We are running BlobToolKit on all public (INSDC registered) eukaryote genome assemblies and making results available in an instance of BlobToolKit Viewer at http://blobtoolkit.genomehubs.org/view/. This genome quality information will be fed back to submitters and to databases to improve the public record. BlobToolKit is also useful for the process of genome assembly from problematic samples, and should become a standard in all laboratories carrying out genome sequencing of new species.

Putting *C. elegans* in its place: genome sequencing of all *Caenorhabditis* species

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All species have a phylogenetic history, a history that is written in their genomes, albeit encoded, scrambled and partially overwritten. *Caenorhabditis elegans*, one of the foremost model organisms, is part of a radiation of >60 species, 55 of which are in culture. We are sequencing the genomes of all available *Caenorhabditis* species to understand how the *C. elegans* model came to be. Currently we have sequenced nearly 50 species, including several from previously unnamed taxa. As well as yielding a species phylogeny with unprecedented resolution, the rich data allow us to explore many facets of *Caenorhabditis* evolution, including genome size (genomes range from 55 Mb to 120 Mb), repeat content (especially mobile elements), genome structure and the pattern of origin of novel genes and gene duplications. We find unexpected evolutionary dynamics in genetic pathways critical to *C. elegans* development. Genome size is correlated with repeat content, and has a strong phylogenetic component coupled with directional change when species shift from male-female to male-hermaphrodite breeding systems. Gene structure changes rapidly, with a general pattern of ongoing loss of introns in the crown *Elegans* and *Drosophilae* species groups. Coupled with the rich forward genetics toolkit for *C. elegans* (which is, in part, transferrable to other related species) and growing interest in *Caenorhabditis* ecology and biogeography, these genomes promise to turn *Caenorhabditis* in to an eco-evo-devo genome model taxon. All our data are available on the caenorhabditis.org GenomeHubs website. We thank colleagues who isolated and cultured new species, and supplied strains and DNA.

GenomeHubs: Distributed genome databasing for neglected organisms

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Sequencing and assembling the genome of a new species is a fundamental step in programmes seeking to understand the diversity of life, and patterns of species distribution and fitness. Despite the effort put into generating gene models and functional annotation for new genomes, it is often difficult to make these available for others to access and reuse. Major database efforts, such as the EBI's ENSEMBL system, capture some, but not all, of the flood of genomes being produced. In addition, ENSEMBL, because it serves the whole community and not just a local interest group, cannot display the richness of work on specific organisms or groups. We have developed GenomeHubs (http://genomehubs.org/), an agile databasing system that is accessible to all research groups. It is based on an ENSEMBL database core, but is otherwise entirely customisable. A GenomeHubs instance can reside on a local computer and offers data search, data browsing, sequence search (through BLAST) and data download tools. As the database is user-controlled, GenomeHubs instances can display multiple assemblies for individual species, and thus act as part of the live research toolkit for labs generating new data. We are also extending GenomeHubs to include new visualisations of genome quality, new ways of interrogating and exploring gene orthology inferences, and also incorporating transcriptome assemblies alongside full genomes.
Representative GenomeHubs databases are linked from the main GenomeHubs website. GenomeHubs code (https://github.com/genomehubs) and instructions (https://genomehubs.gitbooks.io/genomehubs/content/) are freely downloadable.

50 Shades of Red: An analysis of the biodiversity of *Goniobranchus* Nudibranchs

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In the last several years, the family tree of Chromodorididae has been undergoing refinement due to molecular work, indicating new relationships between taxa. The genus *Goniobranchus* is one clade of the Chromodorididae and used to be included within *Chromodoris*. *Chromodoris* was determined to be non-monophyletic and *Goniobranchus* was separated out. Since separation, molecular work to resolve the internal relationships in *Goniobranchus* has not been undertaken. Over half of the proposed species in this genus are still undescribed. Through genetic sequencing, we have sequenced 133 (131 new) *Goniobranchus* specimens representing 39 previously defined species. We used two mitochondrial (COI and 16s) genes and one nuclear gene (H3) to begin to resolve the genetic relationships between *Goniobranchus* species. Phylogenetics was used with Bayesian inference, maximum likelihood, and maximum parsimony analyses to postulate the evolutionary relationships between *Goniobranchus* species.

Ascidian coloniality: from genomes to super-organisms

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Coloniality – a higher level of biological organization with modular and coordinated growth – has evolved multiple times independently in the tunicates, the sister group of vertebrates. What are the most relevant biological factors responsible for the evolution of coloniality? We identify factors acting at three different levels of experimental approaches (i.e. ecological, cellular, and genomic levels) that show an influence on colonial development and life histories. First, by documenting patterns of dominance and growth of solitary and colonial species in artificial substrata, we determined that predation acts as an evolutionary force driving developmental changes favoring coloniality. Second, we compared budding and regeneration in colonial and solitary species of the same tunicate clade. Morphological comparisons of tissues and progenitor cells involved in regeneration and budding support at least two independent events of coloniality in the group, which is supported by a recent phylogenomic study also carried by our group. Third, to understand genomic changes during the transitions to asexual reproduction, clonality, and coloniality, we sequenced the genomes of several colonial species of tunicates de novo, and compared these with available solitary tunicate genomes to target for possible genomic players of budding and asexual reproduction. Our findings have revealed several candidate molecules that may be associated to the solitary-colonial transition, but these require additional validation and experimentation to directly associate them to the evolution of coloniality.

Profiling the genetic diversity and structure of Pocilloporid coral populations along the Red Sea using a population genomics approach - insights from RAD sequencing of *Stylophora pistillata* and *Pocillopora verrucosa*

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Coral reef deterioration, accelerated by global climate change, has increased the importance to investigate the adaptive potential of corals to environmental change. Corals from the Red Sea naturally thrive in warmer and more saline waters than their conspecifics elsewhere. As such, they are candidates to study adaptation to “Future Oceans” conditions. Yet, little is known about the genetic makeup of Red Sea coral populations and how it relates to their success. Using Restriction Site Associated DNA sequencing (RAD-Seq), we evaluate six populations of two species of Pocilloporid corals, *Stylophora pistillata* and *Pocillopora verrucosa*, spread across 13 degrees of latitude in the Red Sea to uncover signatures of positive selection to prevailing environmental conditions. Our results show that the highest genetic diversity is present in the northern populations, highlighting their importance for conservation approaches in line with a suggested coral refuge of this region. Notably, the mode of reproduction was found to align with measures of connectivity between populations: *S. pistillata* (brooder) displayed a stronger population genetic structure than *P. verrucosa* (spawner) with important implications for the designation of conservation areas. Finally, using the available coral draft genome of *S. pistillata*, we identified 139 candidate loci that differ between populations and are candidates for positively selected genes. Our findings suggest adaptation of corals to environmental gradients present in the Red Sea. Current assessment of associated Symbiodiniacaea and bacteria will help evaluate the adaptive potential of the coral holobiont and host genomic factors contributing to microbial community composition.

The genome of the quagga mussel and the evolution of freshwater embryonic osmoregulation

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The quagga mussel *Dreissena bugensis* is one of the most successful and destructive invaders of freshwater environments globally. Having evolved in the freshening waters of the isolated Lake Pannon between 10 and 8.5 million years ago, the native range of contemporary dreissenid species spans the Black and Caspian sea catchments which are remnants of this ancient lake. Invasive populations of the quagga mussel have become established throughout mainland Europe, the British Isles and North America where they have largely replaced the population of their close relative, the equally invasive zebra mussel *Dreissena polymorpha*. In contrast to its osmoconforming marine relatives, the quagga maintains a cellular osmolarity above that of its surrounding medium including during embryogenesis. To identify components of the embryonic osmotic regulatory machinery, we sequenced the genome and transcriptome of the quagga mussel, revealing the upregulation of an aquaporin water channel, a vacuolar ATPase (v-ATPase) subunit I and a sodium hydrogen antiporter during a developmental period defined by the formation of the ‘cleavage cavity’ - a conspicuous fluid filled structure which rapidly and periodically expels its contents to the exterior. The aquaporin belongs to a clade which has independently expanded in freshwater bivalves representing at least four separate freshwater colonisation events. Together with the v-ATPase, aquaporins are integral components of protozoan contractile vacuoles which appear to function analogously to cleavage cavities. The repeated expansion of aquaporins and the formation of cleavage cavities in several freshwater lophotrochozoans suggests that such an embryonic osmoregulatory mechanism may have evolved on multiple occasions.

Phylogenomics without genomes: tales from the understudied

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For many invertebrate groups, de novo genome assemblies have not yet been feasible for a variety of reasons. Inaccessibility of collection localities, small body size, large genome size, and high heterogeneity are just a few of the obstacles to whole genome assemblies. Transcriptomes have been an excellent alternative data source to address phylogenetic questions in many lineages. For example, within Ambulacraria (Echinodermata + Hemichordata), independent transcriptome-based phylogenomic studies have consistently recovered similar relationships. These robust results will help clarify the early evolution of chordate characteristics and have implications for our understanding of major fossil groups including grapolites and somasteroideans. In contrast, the relationship of Xenacoelomorpha to other bilaterians has remained more contentious. Transcriptome-based phylogenomic studies have supported two conflicting hypotheses: Xenacoelomorpha is sister to or within Ambulacraria, or is the sister group to all remaining bilaterians, with two recent phylogenomic analyses supporting the later hypothesis. To address further questions, complete genomes would be invaluable, but have as of yet been intractable in this group, in which the closest reference genome sequence is likely separated by over 600 million years of evolution. Target enrichment and other genome-reduction approaches are cost-effective methods with additional benefits, such as the ability to make use of museum specimens. Demonstrating the utility of genome reduction methods for taxa without reference genomes, we have generated an effective target capture probe set for poylplacophoran molluscs. These approaches and other methodological innovations have the promise to significantly advance our understanding of the animal tree of life.

Binning Facilitates Reconstruction of Nuclear Host Genomes in Complex Cnidarian Holobionts

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Many cnidarians, including stony corals, engage in complex symbiotic associations, comprising the eukaryotic host, photosynthetic algae, and highly diverse microbial communities – together referred to as holobiont. This taxonomic complexity makes sequencing and assembling coral host genomes extremely challenging. Therefore, previous cnidarian genomic projects were based on symbiont-free tissue samples. However, this approach may not be applicable to the majority of cnidarian species for ecological reasons. We therefore evaluated the performance of an alternative method based on sequence binning for reconstructing the genome of the stony coral *Porites rus* from a hologenomic sample, and compared it to traditional approaches. Our results demonstrate that binning performs well for hologenomic data, producing sufficient reads for assembling the draft genome of *P. rus*. An assembly evaluation based on operational criteria showed comparable results to symbiont-free approaches in terms of completeness and usefulness, despite a high degree of fragmentation in our assembly. In addition, we found that binning provides sufficient data for exploratory *k*-mer estimation of genomic features, such as genome size and heterozygosity. Binning constitutes a powerful approach for disentangling taxonomically complex coral hologenomes. Considering the recent decline of coral reefs on the one hand and previous limitations to coral genome sequencing on the other hand, binning may facilitate rapid and reliable genome assembly. This study also provides an important milestone in advancing binning from the metagenomic to the hologenomic and from the prokaryotic to the eukaryotic level.

New tools for old problems: Population genomics and biogeographical inference of the *Stygocapitella subterranea* cryptic species complex (Annelida: Parergodrillidae)

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Many interstitial species were first described as widely distributed, often cosmopolitan or amphi-oceanic. These accounts contrasted descriptions of a sedentary life style and the general absence of pelagic dispersal stages, an inconsistency that became known as the meiofauna paradox. Recent application of genetic data unravelled the occurrence of multiple cryptic species (morphologically indistinguishable species) often with restricted distributions. Nonetheless, our knowledge is limited due to the usage of only a few specific genetic markers, which do not provide a good resolution to understand barriers to gene flow in the sea.
Here, we describe a methodology for whole-genome amplification coupled with Double Digest Restriction-site-Associated DNA sequencing (ddRADseq) applied to population genomics of interstitial invertebrates. By increasing the input DNA concentration via whole genome amplification and applying genome digestion (ddRADseq), we were able to generate thousands of informative markers. Using data generated from 192 specimens, we study the evolutionary history of the cryptic species complex of *Stygocapitella subterranea*. We report the occurrence of multiple cryptic species, some co-occurring in sympatry. The obtained dataset provides fine-scale biogeographical resolution and allows for demographic inferences to unravel the evolutionary history of these species. We discuss the potential of new approaches to generate population genomics datasets to tackle long-standing questions in invertebrate biology.

Disentangling a taxonomic conundrum: Cryptic species as separate evolutionary lineages

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The development and application of DNA sequencing in biology uncovered unrecognized diversity within previously established species – i.e. 'cryptic species'. At first sight, the unveiling of cryptic species suggested the occurrence of taxonomic artefacts and generated a discussion focusing on revalidation of taxonomic practices. Nevertheless, another aspect of cryptic species is that they might result from evolutionary processes such as selection for a conserved morphology. In this poster we present a two-step framework to delimit cryptic species complexes as well as various evolutionary processes, which can result in cryptic species, including morphological stasis (i.e. the conservation of a morphological bodyplan), recent speciation, parallel and convergent evolution. We suggest that cryptic species, which evolved due to the process of stasis, could provide a link to paleontological stasis and conclude by discussing implications of cryptic species to evolutionary biology, ecology and systematics.

The state of knowledge and resources for marine invertebrate genomic research

Jonathan A. Coddington

Smithsonian Institution National Museum of Natural History

Genomic science is revolutionizing biodiversity research. This talk will summarize what is known about marine invertebrate biodiversity in terms of the literature, distribution, biological knowledge, genomics, and genetic resources, identify strategic gaps, and outline criteria and goals for priorities and to advance marine invertebrate genomics.

Pocillopora damicornis immune gene expression patterns in response to antibiotics treatments, heat stress and immune stimulation with bacterial lipopolysaccharide

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Reef-building corals establish diverse symbioses with dinoflagellate algae, bacteria, and micro-eukaryotes that benefit the health of the coral holobiont. Climate change is causing a global collapse of coral reef ecosystems, as high sea surface temperatures disrupt the stability of symbioses within the coral holobiont, causing coral bleaching, disease and death. Activation of coral innate immunity has been observed during the coral stress response, which may also be affected by the composition of the coral bacterial community. To disentangle patterns of immune gene expression driven by heat stress and bacterial community disruption, *Pocillopora damicornis* corals from Kenting National Park in Taiwan were subjected to multiple treatments with antibiotics (ampicillin and streptomycin), heat stress, and immune stimulation with bacterial lipopolysaccharide (LPS). Upregulation of genes involved in immune recognition and signaling pathways were observed in treatments with antibiotics and LPS, suggesting that antibiotics treatment causes a dysbiosis in the coral bacterial community that drives immune inflammation. Additionally, weighted gene co-expression network analysis revealed numerous co-expressed gene modules that were significantly correlated to treatment conditions. At least 3 modules that were positively correlated to LPS treatment and negatively correlated to heat stress were also enriched in genes related to innate immunity, cellular defense and cell-cell signaling including Toll-like receptors (TLRs), c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) pathways. Altogether, these results corroborate reports of coral immune suppression during heat stress and suggest that stability in the coral bacterial microbiome is essential for coral immune function and homeostasis under future climate scenarios.

Resolving deep nodes in the gastropod phylogeny

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One of the most diverse animal groups, gastropods comprise over 60,000 species of marine, terrestrial and freshwater snails and slugs. Despite an impressive diversity of species and forms, the phylogeny of the clade is still unresolved, with previous transcriptomic analyses based on concatenated matrices having left critical nodes to be resolved at the deep scale. We present 17 new transcriptomes of Patellogastropoda and Neritimorpha, which had been poorly sampled with genomic-scale data so far, now covering all major gastropod lineages with good taxonomic sampling. We produce the first coalescent-based approach to infer the species tree of gastropods. We further use maximum likelihood and Bayesian analyses of aminoacid and dayhoff matrices and explore more complex models of sequence evolution. The dataset has 56 ingroup terminals and two matrix sizes, a large set with 1059 genes and a small and more complete set with 149 genes. All analyses with the large dataset and most with the small dataset resulted in the same topology with full or very high support, placing neritimorphs (*Nerita* and relatives) as the sister group of apogastropods (whelks, moon snails, nudibranchs and others), while patellogastropods (true limpets) are recovered as the sister clade of vetigastropods (abalones, turban snails, keyhole limpets and relatives). Gene tree conflict in the small dataset left the deep nodes unresolved in the coalescent-based analysis. Overall, different analytical methods resulted in a stable and well-supported topology, emphasizing the importance of filling gaps in taxon sampling in phylogenomics.

From the shallows to the abyss: Utilizing phylotranscriptomics to characterize crustacean visual opsin diversity

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Crustaceans have successfully colonized a range of habitats in the ocean – from the epipelagic to the abyssal zone. Some species exhibit entirely pelagic life histories, while others are benthic or epibiotic, colonizing sessile invertebrates like coral and sponges. Moreover, some deep-water genera are bioluminescent, capable of producing light for purposes of predator avoidance and communication. These environmental and ecological differences can have notable impacts on sensory mechanisms, including an organism's visual capabilities. Crustaceans therefore present an ideal system to investigate the interplay between adaptation and the environment, which can strongly influence the evolution of visual systems. In this study, we investigated the diversity and evolution of crustacean visual opsins – light-sensitive pigment proteins that influence spectral tuning and initiate phototransduction cascades. RNA was extracted and sequenced from 23 crustacean species primarily inhabiting mesopelagic or deep-benthic environments (~200 – 4000 m). *De novo* transcriptomes were assembled from high-throughput sequencing data and analyzed via phylotranscriptomic methods to characterize opsin diversity. Diversification was further explored in terms of differing environmental niches (i.e., mesopelagic, benthic, epibiotic) and bioluminescent capabilities. We recovered a range of putative visual opsins (1 – 5) for each species corresponding to several spectral clades. Opsin diversity was highest in pelagic bioluminescent crustaceans as well as deep-benthic species which are known coral associates. Our findings also indicate that crustaceans possess unique visual pigments that do not cluster with the opsins of other arthropods (Insecta). Lastly, this study illustrates the strength of employing next-generation sequencing and phylogenetic approaches towards the study of deep-sea sensory systems.

Transcriptomic changes during regeneration in marine sponge *Cinachyrella* cf *cavernosa*

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The sessile lifestyle of marine sponges exposes them to the environmental forces which may cause wounds and stress. Hence, regeneration becomes a vital process in these organisms. The advents of high-throughput sequencing and transcriptomics have widened doors for molecular investigation of regeneration process. This study assesses gene expression during different stages of regeneration in demosponge *Cinachyrella* cf *cavernosa*. Specimens of this slow growing tetillid sponge were inflicted with wounds in laboratory aquaria. Control tissue, regenerating tissue, and completely regenerated tissue were utilized for transcriptomic analysis. The sequences were assembled using Trinity, annotated, and compared among the three samples. In the pooled assembly, 102,443 Unigenes were reported. Total 65,587 coding DNA sequences (CDS) were found, out of which 37,145 had BLAST hits. The genes associated with cell turnover expressed in the highest number in control tissue sample. Their number reduced in regenerating tissue sample with resurgence in the completely regenerated tissue sample. The observed gene expression pattern may be due to the shift from homeostasis maintenance in control tissue to wound healing in regenerating tissue. WNT-6 gene from WNT pathway was maximally expressed in regenerating tissue and the expression of this gene reduced with the progress of regeneration, signifying the involvement of WNT pathway in early stages of regeneration. In contrast to other metabolism related genes, the number of genes associated with lipid metabolism increased with progressing regeneration, indicating their role in stress management. This study advances our understanding of transcriptomic changes related to regeneration process in poriferans.

Evaluating adaptive potential and identifying markers of thermal tolerance in a Great Barrier Reef coral *Platygyra Daedalea*

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Coral bleaching events resulting in subsequent coral mortality are predicted to increase in frequency and severity due to climate change. Corals must adapt to survive in these warming oceans. Selective breeding of naturally tolerant corals is one of several genetic mechanisms proposed to increase thermal tolerance of coral populations. However, little data is available for predicting cross generation genomic adaptive responses in coral. We need to understand which populations have the capacity to adapt to warming temperatures and which genetic variants confer increased thermal tolerance to support proposed conventional and novel reef management actions. I will describe a laboratory based heat selection experiment where we tracked survival of individual coral larva to determine if survival in acute heat stress conditions could be passed from parent to offspring in one coral species. The heritability of thermal tolerance in *Platygyra daedalea* from the Great Barrier Reef (GBR) is 0.66 and suggests that this population has the strong capacity to adapt to increased temperatures. We identified a total of 1,069 genetic markers associated with thermal tolerance overall with 34-336 markers in each family. An average of two to three markers were identified in each comparison of marker overlap between groups of three experimental families indicating that those specific genomic regions are important for thermal adaptation of *Platygyra daedalea*. These results underpin the importance of understanding selection and the genetic architecture of selected traits in the context of conventional and novel management actions.

Integrated Genomic Resources for a Temperate Model of Cnidarian-Algal Symbiosis

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Corals support diverse reef ecosystems, but they are declining because of rising ocean temperatures which disrupt symbiosis between corals and diverse algal symbionts *Symbiodinium*. The symbiont types hosted by corals contribute to thermal tolerance of the holobiont, yet the mechanisms underlying maintenance and loss of symbiosis remain poorly understood. These studies are difficult in coral because of their thermal sensitivity, restricted distributions, and cryptic variation in symbiont types. We are building genomic resources for a temperate anemone *Anthopleura elegantissima* which provides a model for symbiosis and environmental stress tolerance. *A. elegantissima* presents variation in visually distinct symbiotic states, associating with a green alga *Elliptochloris marina*, a dinoflagellate *Symbiodinium* sp., and occurs in an aposymbiotic state. Maintenance of associations in the thermally dynamic intertidal zone and across a wide latitudinal range is a contrast to the more thermally sensitive associations in corals. To establish genomic resources for this model, we collected anemones across their geographic range. We have constructed a draft genome assembly for *A. elegantissima* from the chosen accession. Here we describe genome assembly and annotation, genomic patterns of population differentiation across the US west coast, and our efforts to develop and integrate a genetic linkage map, transcriptome, and genome assemblies.

Draft assemblies of a bryozoan and a chaetognath with Illumina and Oxford Nanopore data sets

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Despite the advent of big data and Next Generation Sequencing approaches applied to non-model organisms in the last years, still almost half of the animal phyla have never been sequenced at the genome level. In this talk, I will discuss our most recent efforts to sequence and assemble the genome of two neglected animal phyla: bryozoans and chaetognaths. In collaboration with Kira Treiberg, Bob Woollacott and Gonzalo Giribet (Harvard University), we sequenced the genome and transcriptome of *Bugula stolonifera* (Ryland, 1960) (Bryozoa, Gymnolaemata) with the Illumina platform (both pair-end and mate-pair reads). Due to the limited biological material, we optimized a whole genome amplification protocol based on single cell methodologies that allowed us to sequence both the genome and transcriptome from a single zooid. The preliminary draft genome shows a high percentage of BUSCO completeness. We did not detect the presence of any endosymbionts, confirming previous findings suggesting that they are inherited vertically. Moreover, in collaboration with Juan Hofer (Universidad Austral de Chile) and Toni Gabaldón (Center for Genomic Regulation), we sequenced the genome of *Serratosagitta tasmanica* (Chaetognatha), this time with Oxford Nanopore and Illumina technologies. In this presentation, I will discuss the methodologies, technical problems encountered and tips both at the wet lab and bioinformatic analysis to deal with this type of samples and datasets.

Assembling genomes into complete chromosomes using chromosome conformation capture: the case of the bdelloid rotifer *Adineta vaga*

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Genome scientists commonly turn a blind eye to heterozygosity by aiming to reconstruct a non-redundant haploid genome. As a result of this methodological bias, most short-read assemblers available to date are incapable of resolving diploid or polyploid genomes. Theoretically, the use of third-generation sequencing reads of great lengths (such as PacBio andNanopore reads) and/or the long-distance information provided by chromosome conformation capture (3C) should solve the problem of diploid/polyploid genome assembly and produce chromosome-scale, haplotype-specific assemblies, butfully resolved heterozygous genomes are still extremely rare in the literature. As a test of the potential of these approaches to deliver "perfect" assemblies, we turned to the reasonably sized genome of the bdelloid rotifer *Adineta vaga* (expected size: 244 Mb). Bdelloid rotifers are famous for their tens of million years of evolution in the apparent absence of meiotic sex (only females have ever been observed, and they produce eggs clonally via mitotic parthenogenesis). In 2013, we published a first diploid (actually, tetraploid) draft genome of *Adineta vaga*; here we present the final assembly of this genome into its twelve constituent chromosomes using a combination of short reads, long reads and chromosome conformation capture.

Towards the genetic prediction of bleaching response in corals

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Coral reefs are facing a global crisis, as increasing seawater temperatures and other environmental stressors are triggering mass bleaching episodes. Within populations, however, there is considerable phenotypic variation in the bleaching response. In the coral *Acropora millepora* on the Great Barrier Reef (GBR), this variability has been shown to be partly heritable, i.e., due to segregating genetic variation. Unfortunately, it is unknown which individuals are more likely to bleach and therefore where to prioritize conservation efforts. To address this urgent problem, we propose to perform phenotypic prediction in corals by conducting a genome-wide association study (GWAS) of bleaching response, borrowing on methods widely used in agriculture and human genetics. To this end, we first constructed a highly contiguous *de novo* assembly of the *A. millepora* genome. We further resequenced multiple whole genomes of individuals collected from 12 locations on the GBR in order to characterize genome-wide patterns of linkage disequilibrium and genetic diversity. Using these 48 genomes, we characterized the demographic history of sampled *A. millepora* populations: consistent with previous studies, we infer high levels of migration between distant locations, but also find evidence that specific inshore reefs have been reproductively isolated for a long time, possibly suggesting the presence of cryptic species. We also find evidence for drastic changes in effective population sizes over evolutionary timescales. Population parameters estimated from these data indicate that large-scale, low-coverage sequencing and genotype imputation procedures should be feasible in this species, setting the stage for genome-wide mapping studies of bleaching and other phenotypes.

Identifying the genetic mechanisms underpinning the patterning of antenniform legs in arachnids using a differential expression approach in whip spiders (Arachnida, Amblypygi)

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The specialization of legs toward a sensory function in some arachnids represents an interesting functional convergence with the insect antenna. Nonetheless, nothing is known about the genes patterning antenniform legs, much less about the genes that differentiate legs of arachnids more generally. To investigate the patterning of sensory legs, we studied embryogenesis of the whip spider *Phrynus marginemaculatus*, as an exemplar of an order where the first leg pair (L1) is modified into elongate, antenniform appendages. We generated leg-specific transcriptomic sequence data of L1 (antenniform) versus L2-L4 (non-antenniform) appendages dissected from deutembryos. Reads were mapped onto the first embryonic transcriptome of an amblypygid and differential gene expression analysis was conducted using DESeq2, with the aim of circumscribing candidate genes involved in leg antennification (i.e., genes differentially expressed in L1, in comparison to L2-L4). To investigate the specification of L1 in a lineage that has typical walking legs on this segment, we selected the spider *Parasteatoda tepidariorum* and generated an L1-to-pedipalp homeotic transformation via RNAi-mediated knockdown of the Hox gene *Dfd-1*. Loss-of-function embryos displayed two pairs of pedipalps and three pairs of legs. Using the same transcriptomic approach as for the whip spider, we compared gene expression in the spider phenocopies with L1-to-pedipalp transformations versus wild type embryos. By discovering the intersection as well as the non-overlap of the differentially expressed genes from both species, we establish here a list of candidate genes that may be uniquely involved in patterning an antenniform appendage, and generally involved in patterning L1 across arachnids.

High quality genome assembly of the octocorallian *Corallium rubrum*

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The Mediterranean red coral, *Corallium rubrum* (Cnidaria, Anthozoa, Octocorallia, Alcyonacea, Scleraxonia) is an emblematic representative of the "precious corals". It has been used in the jewelry industry since Greek Antiquity; its value is inherently linked to the properties of its calcified skeleton, which is polished into red gemstones. Indeed, although most octocorallians produce magnesium calcite biominerals in the form of sclerites (minute, spiny skeletal elements), scleraxonians also produce an axial pigmented skeleton. In order to gain insight into the mechanisms underlying calcification in the red coral (a comparative model for calcification with scleratinian corals) we have assembled the *C. rubrum* genome, the first octocoral genome sequenced to our knowledge. Our assembly uses illumina short reads and PacBio long reads libraries. Our original assembly pipeline is based on the MaSurCa hybrid genome assembler and makes use of RNAseq and long mate-pair libraries for additional scaffolding. Overall, most of our genome assembly metrics overtake those of already published cnidarian genomes, especially with respect to "Ns", which represent less than 1% of our total 600 Mb assembly.

Molecular paleobiology reveals the deep evolution of Ecdysozoa

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Ecdysozoa is a superclade uniting eight phyla of protostome animals, including the most diverse and most abundant of all animals – the arthropods and nematodes respectively. Whilst phylogenetically robust, the internal topology of Ecdysozoa remains unresolved. Of particular concern is the monophyly of the subclade Cycloneuralia, which remains in common use despite generally lacking molecular support. Furthermore, the timings of ecdysozoan lineage divergences in geological time are poorly constrained, with molecular clock analyses suggesting ecdysozoans diverged in the Precambrian, despite ecdysozoan fossils not being identifiable until early Cambrian. This lack of clarity in early ecdysozoan evolution cascades onto adjacent branches of the Tree of Life and presents a considerable constraint on interpreting the origins of the Phanerozoic animal dominated biosphere, and the nature/existence of a Cambrian evolutionary ”Explosion”. To address these obstacles, we assembled a large phylogenomic dataset comprising up to 229 genes for 66 ecdysozoan taxa (and 14 outgroups across Metazoa). In particular we added new data for key taxa with poor or no molecular data (Loricifera, Kinorhyncha, Nematomorpha, Heterotardigrada) and applied Bayesian methods to infer the phylogeny and divergence times of the eight ecdysozoan phyla. Our phylogenetic analysis support a sister group relationship between nematoids and panarthropods (rendering Cycloneuralia paraphyletic), and sensitivity tests on our fossil-calibrated relaxed molecular clock analyses support the hypothesis that the high order bilaterian groupings had already diverged before the Cambrian, but the extent of the lag between the genetic isolation of the Ecdysozoa lineage and its representation in the fossil record is still unclear.

Using RNA-Seq to elucidate the phylogeny of Polycladida (Platyhelminthes), a flatworm clade with diverse life histories

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Flatworms are among the most diverse invertebrate phyla, with over 100,000 parasitic and free-living species**.** Polycladida, an order of predatory marine flatworms, exhibits direct, intermediate, and indirect development and a diversity of larval morphologies. Because life history strategies are important components of fitness, understanding the evolution of modes of development and larval morphology can help us comprehend the complexities of adaptation. However, the lack of a highly supported, well-resolved phylogeny has long prevented this work in Polycladida. This order has traditionally been divided into two sub-orders based on multiple morphological characters, including the presence (Cotylea, ~350 species) or absence (Acotylea, ~450 species) of a ventral adhesive structure. Due to morphological homogeneity and insufficient molecular data, deep divergences among taxa within Polycladida remain poorly understood. To improve phylogeny inference for this clade, we generated RNA-Seq data for 20 species of polyclads and combined these data with transcriptome data from fifteen additional in- and out-group taxa from the Sequence Read Archive for phylogenetic inference. We next reconstructed ancestral life history characters among the lineages in our tree in order to discern where particular development modes and larval forms originated. Our results provide a well-supported preliminary hypothesis for early divergences within Polycladida, including support for the two sub-orders Cotylea and Acotylea. Further, this phylogenetic hypothesis offers us a more robust framework for understanding the evolution of development in polyclad flatworms. This work represents an important step in our comprehension of how life history evolution relates to adaptation among flatworm clades.

Differential gene expression profiles of the bearded fireworm, *Hermodice carunculata*, in hypoxic conditions

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The bearded fireworm, *Hermodice carunculata* (Family Amphinomidae: Phylum Annelida), is a widespread mobile corallivore in the Atlantic Ocean. Due to its broad geographic distribution, abundance, and environmental tolerances (including low oxygen), it may serve as a model organism for hypoxia studies, which are increasingly important with the projected escalation of hypoxia zones in the future. Molecular responses of *H. carunculata* to hypoxic conditions were investigated to determine the dissolved oxygen (DO) level at which they express hypoxia response genes. Five bearded fireworms were exposed to one of two levels of moderate hypoxic conditions in 40-liter tanks for seven days: 2.5 (± 0.25) mg/l and 4.5 (± 0.25) mg/1. A third tank containing five fireworms were maintained at normoxic conditions for the duration of the study. No reference genome exists for the species; therefore, a combined reference transcriptome was generated from multiple specimens and utilized to align the individual RNAseq transcriptomes for differential gene expression analysis with EdgeR®. Pairwise comparisons revealed up-regulation of key hypoxia and stress response genes and down-regulation of metabolic pathway genes in the worms under hypoxia. Differences in gene expression were also noted between the two experimental groups, indicating the DO levels chosen were at intervals distinct enough to invoke differing responses. The results allow us to infer the threshold DO level for hypoxic response in this abundant and environmentally tolerant coral predator and to predict downstream responses.

Identification of protein domains on putative ancestral subgenomic regions associated with genes of the immune system in the subphylum Tunicata using as reference genes of human cell-lines previously mapped on *Botryllus schlosseri.*

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The adaptive immune system (AIS) is a complex and an important system that has been exclusively found in the subphylum Vertebrata. Nevertheless, understanding its origin and evolution is still the topic of many ongoing projects. Most of these studies have been focused on exploring the immunological diversity in model organisms such as primates and rodents but researches in non-model organisms as marine invertebrates remain poorly explored. With this in mind, we have chosen to work with Tunicata, because of their unique phylogenetic position and their diversification event happening before the immunological Big Bang. Tunicates are then of utmost important to trace-back genomic trails which could help to understand AIS origins. By extending the approach described by Voskoboynik in 2013, we developed a computational strategy to detect protein domains and subgenomic regions associated with putative ancestral genes of the immune system. Surprisingly in our results, besides many important detected domains, immunoglobulin (Ig) and Leucine-rich repeat (LRR) domains were fairly detected across tunicates and vertebrates. Hundreds of domains were identified to be distributed across vertebrates, tunicates and a cephalochordate as well as putative subgenomic regions associated with putative ancestral genes of the immune system in the subphylum Tunicata. These set of genomic locations could embed candidate genes to be validated in the future by molecular experimentalists.

Does evolution of generalism in *Bemisia tabaci* relate to positive selection in cytochrome P450 enzymes?

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The phloem feeding whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) is a cryptic species complex containing more than 28 distinct species. The complex is generally considered polyphagous, but our true knowledge on the host range of the species is patchy. An extensive literature survey indicated that only three species can be considered as “true” polyphagous, while the others can be roughly divided into two groups based on their characteristics of oligophagy. Our dating of the complex shows divergence in the youngest species around 0.5-1.5 Mya. This suggests that the dispersal of the *B. tabaci* can be related somehow even to the human agriculture effect. We hypothesized that the observed differences in host plant adaptation between the species may be driven by evolved differences in their detoxification “tool-kit”, providing them differential ability to detoxify defensive secondary plant metabolites that interfere with their survival. We focused on the cytochrome P450 monooxygenase gene family, which is known for its ability to metabolize a wide range of endogenous and exogenous compounds. We screened for mutations that provide orthologous genes with an advantageous altered function that may be positively selected P450 genes were recovered from eight species, representing major groups of the complex. Using bioinformatics tools, a P450 phylogenetic tree was built and natural selection tests were carried out on each enzyme (omega values calculation) together with modeling of the structure. In some of them, there are sites showing positive selection. Candidate genes with high potential to be involved in the species host plant adaptation were identified.

Evolution on Ice: 'Omic insights into Molecular Adaptation in Antarctic Sponges

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Animals in the Antarctic seas have adapted to some of the most challenging conditions found anywhere on Earth. Temperatures ranging between 0 and -1.8°C and a food supply which fluctuates widely render their survival difficult. Nevertheless, species have found the means to thrive in such conditions. Sponges are particularly important members of Antarctic ecosystems, but to date our knowledge of how they endure these temperatures is limited at best, especially at a molecular level. We aim to identify the mechanisms by which sponges have adapted to such extreme environments by contrasting congeneric species pairs adapted to vastly differing thermal environments. These aims are being accomplished using transcriptomic and genomic sequences from the genera *Axinella*, *Mycale* and *Phorbas*. These are abundant in the Antarctic, Caribbean and Mediterranean, and play essential roles in the benthic ecosystems in which they are found. Particularly, we have sequenced multiple transcriptomes from 10 target species, as well as the genomes of *Mycale acerata* and *Mycale laevis,* and aim to supplement our "omic" work with targeted *in situ* and functional experiments. Using this data, we have performed a number of tests for selection (particularly in Hyphy/CODEML) and identified genes with multiple lines of evidence for positive selection, including a number of phylogenetically well-conserved "housekeeping" genes. We have also analyzed differential gene expression and content. With this data, we can state which genes are vital in cold conditions, and when adaptive molecular mechanisms have been used broadly, convergently, or in vastly varying ways across sponge and animal phylogeny.

Population dynamics and outlier analysis of the sea urchin, *Echinometra sp.* EZ

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Climate change has resulted in warming of coastal aquatic habitats around the world at almost every latitude, threatening ecosystems with a significant loss in biodiversity and potential collapse. It is essential that we understand how organisms will respond to changing environmental conditions, particularly with respect to keystone species where the impacts of climate change could have far reaching consequences. Sea urchins of the genus, *Echinometra,* are keystone herbivores found across the Indo-Pacific and play a significant role in the health and dynamics of reef ecosystems as major bioeroders. Genomic analysis of species living in natural extreme environments can help identify how populations will persist through future climate change. We set out to investigate population structure and targets of selection in *Echinometra sp.* EZ through RADseq of samples from seven populations in the Gulf of Oman (summer maxima ~30-32°C) and the neighboring thermally-extreme Persian/Arabian Gulf (summer maxima ~35-37°C). Further, we have assembled a genome using Illumina short read technology that will serve as a resource for aligning our RADtags. Population genetic analyses identified the presence of two populations (Structure; K=2), each associated with either Gulf, although population structure was weak (F_{ST}=0.0059). Interestingly, outlier analysis identified genes under selection involved in transcription regulation and methylation. The results generated from this study will provide crucial data on a keystone species experiencing extreme environmental conditions, and fill a critical knowledge gap by providing mechanistic insights into how species will respond to climate change.

Revisiting contemporary hybridization between Caribbean Acroporids

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Reef-building corals are currently threatened by rapid changes in local and global stressors, and hybridization offers a potential shortcut for rapid adaptation and evolutionary rescue in these species. The sympatric corals *Acropora palmata* and *A. cervicornis* form the hybrid, *A. prolifera*, whose abundance has continued to increase while the parental species decline. Previous work indicates that weakened prezygotic isolation mechanisms in *A. cervicornis* but not *A. palmata* could allow for continuous unidirectional gene flow between the two species. Furthermore, asymmetric introgression from *A. palmata* to *A. cervicornis* has been recorded in three nuclear loci. In contrast, we found evidence for bidirectional introgession across three hybrid zones although the frequency of hybrids and backcrosses differs across the range. Genome assemblies of *A. palmata* and *A. cervicornis* were compared to other corals to identify orthologs uniquely shared by the Caribbean Acroporids. Genomic sequence data from the two parental species and their hybrids was used to further characterize the patterns of genomic synteny, divergence and introgression across hybrid zones. We identified 49,076 variants fixed between the parental species as well as genomic regions with exceptionally high differentiation among parental species. In particular, fixed amino acid differences between these two species were enriched in proteins associated with development, cellular stress response, and the host's interactions with associated microbes. Combined, these approaches elucidate genomic hotspots of introgression with implications for how hybridization may shape adaptation in these important foundation species across the Caribbean and North-West Atlantic.

Phylogeny and Function of a Newly-Discovered Coral Parasite within "*Candidatus* Marinoinvertebrata"

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A novel nutrient-responsive bacterial parasite (order Rickettsiales) is found in several coral host species and is associated with reduced host growth and increased disease susceptibility. Phylogenetic analysis using the full-length 16S rRNA region yielded sufficient statistical evidence to define the clade of Rickettsiales containing this OTU and other sequences from marine invertebrate hosts as a new genus, which we named "*Candidatus* Marinoinvertebrata." Functional annotation revealed a highly-reduced genome lacking genes for necessary compounds and amino acids. The dependency of the parasite on the host for N-rich metabolites prevents uncontrolled growth of the parasite except in nutrient-enriched conditions. We uncovered a complete NtrY-NtrX two-component system that is involved in detecting extracellular nitrate levels, suggesting this organism can sense and respond to nitrogen levels, despite its inability to metabolize nitrogen. Additionally, we observed the presence of Tlc, an antiporter found only in Rickettsiales and Chlamydia that provides the microbial cell with host ATP in exchange for ADP and therefore drains the host of energy, and a Type IV secretion system (T4SS), which may be involved in host attachment and pathogenicity. We hypothesize that this obligate intracellular parasite grows and overwhelms host immune capabilities in response to nutrient stimulation. Although the presence of a T4SS suggests this species can infect and manipulate the host, the association of this organism with disease is likely due to its ability to weaken and eventually kill host mucocytes by scavenging their nutrients and energy, leading to decreased mucus production and therefore increased vulnerability to opportunistic pathogens.

Confessions of a Ph.D. candidate: lessons learned from phylogenomics on heterobranch gastropods

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Phylogenetic relationships within Heterobranchia (Gastropoda) have been researched extensively, but have been difficult to resolve because of the independent or parallel evolution of many morphological features, blah, blah, blah. Despite over a decade of experience with molecular techniques, I found working with RNA from heterobranchs to be particularly challenging (a major pain). The most challenging part, however, seemed to be the lack of resources for working on non-model organisms, or sources to check my results against. Are my results typical? Do others struggle with these same problems? Is it me, or is it the samples? Preservation? In this talk, I will identify challenges I faced in extracting and sequencing various heterobranch taxa after extracting hundreds of tissue samples, mostly preserved in RNAlater, but also flash-frozen in some cases. While our university's molecular core is top notch, they are constantly surprised by the variety of taxa our lab brings through, which hints that their expertise is not always sufficient to help us with our taxon-specific challenges. This also applies to vendors, who are far more accustomed to helping customers working on model taxa. While I did not assess these methods systematically, certain patterns emerged from experience and certain methods seem to perform better than others. The intention of this talk is to hopefully start a dialogue for graduate students, or anyone that works on transcriptomics, to share methods, and perhaps achieve catharsis.

Phylotranscriptomics sheds new light on the sea urchin tree of life

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Echinoidea is a clade of marine animals including sea urchins, heart urchins, sand dollars and sea biscuits. Found in benthic habitats across all latitudes, echinoids are key components of marine communities such as coral reefs and kelp forests. A little over 1,000 species inhabit the oceans today, a diversity that traces its roots back at least to the Permian. Previous phylogenetic analyses based on both molecular and morphological data have had limited success at resolving the deepest nodes of the echinoid tree, and their disagreement over the positions of a number of clades remains unresolved. We performed *de novo* sequencing and assembly of 17 transcriptomes to complement available genomic resources of sea urchins, and produce the first phylogenomic analysis of the clade. Multiple methods of probabilistic inference recovered identical topologies, with virtually all nodes showing maximum support. In contrast, the coalescent-based method ASTRAL-II resolved one node differently, a result apparently driven by gene-tree error induced by evolutionary rate heterogeneity. Regardless of the method employed, our phylogenetic structure deviates from the currently accepted classification of echinoids, with neither Acroechinoidea nor Clypeasteroida being monophyletic. We obtain a novel phylogenetic position for the enigmatic deep-sea echinothurioids, as well as corroborate results from previous molecular studies that had not recovered sand dollars and sea biscuits as sister clades. Finally, we demonstrate the strength and distribution of phylogenetic signal throughout the genome for novel resolutions of these lineages, and find no evidence that they might be driven by systematic biases.

Are ultraconserved elements an informative phylogenetic marker for reconstructing deep molluscan phylogeny?

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Although recent phylogenomic studies employing hundreds of nuclear protein-coding genes have greatly improved understanding of mollusc class-level phylogeny, placement of some lineages such as Scaphopoda and Monoplacophora remain unsettled. We investigated whether ultraconserved elements (UCEs), putative regulators of animal gene expression with very low rates of sequence evolution, could be used as an alternative to nuclear protein-coding genes. To this end, we downloaded publicly available genomes from ten molluscs (six bivalves, three gastropods, and one cephalopod) and five outgroup taxa (two annelids, one brachiopod, one phoronid, and one nemertean) and screened them for UCEs following established approaches as implemented in PhylUCE. This approach identified 4,759 UCEs shared among at least ten taxa and 325 shared across all fifteen taxa. Using a test set of the 529 UCEs with no less than 70% data completeness, we assembled and analyzed a matrix with 142,817 nucleotide positions. Maximum likelihood analysis in RAxML using the GTR+G4 model yielded a tree with strongly-supported relationships that are largely consistent with the current understanding of molluscan evolution. Thus, these preliminary results indicate that this approach has promise for resolving lingering debates about mollusc class-level phylogeny. To this end, we are expanding this dataset to include other public datasets and new genomic data from representatives of three other molluscan classes.

Does chromatin diminution affect invertebrate genome assembly?

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The genomics of non-model invertebrates remains challenging. Every step from sampling to final genome annotation has unexpected complications. We sequenced two marine invertebrate genomes: the Manila clam (*Ruditapes philippinarum*) genome, and the moon jellyfish (*Aurelia aurita*) genome, using both Illumina and PacBio platforms. We failed to assemble those genomes using all available conventional assemblers or more sophisticated assembly pipelines. Initially we explained this by very high heterozygosity rate and highly repeated genome composition. However during the stage of coverage-wise polishing of PacBio-only assembly by Illumina reads we observed many cases of complex structural variants that cannot be explained by diploid heterozygosity or repeats. After manual check of these regions we found the presence of two types of similar sequences but different in read coverage. We developed a software that analyzes all forks in de Bruijn graphs constructed from Illumina reads and we found that both the clam and the jellyfish genomes additionally to the main 20x coverage have many fragments with a 4x coverage. We manually checked the difference between 20x and 4x fragments finding that they are very similar in sequence but do not have classical heterozygosity difference. We found multiple indels that cannot be explained by Illumina errors or observed natural heterozygosity or by presence of DNA from gametes. We hypothesize that these two genomes are subject to chromatin diminution—a chromosomal fragmentation, followed by the elimination of part of the chromosome during mitosis. We have developed a specific assembly pipeline to trying to solve this issue.

Effects of light on coral growth, morphology, and Symbiodiniaceae communities in the coral genus *Pavona*

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Coral reefs are at risk due to rapidly changing marine environments, a consequence of multiple anthropogenic stressors. While the majority of corals are declining, some genera are bucking this trend and increasing in abundance, including *Pavona*. The most common species*, P. varians*, is a widely distributed habitat-generalist. This coral is aptly named exhibiting diverse morphologies, and field observations indicate, extensive phenotypic plasticity. This species harbours diverse communities of the photosynthetic dinoflagellate Symbiodiniaceae, and symbiont communities can differ across environmental gradients. It’s possible that this morphological and microbial plasticity would enable *P. varians* to acclimatize to rapidly changing marine environments, as they get warmer and more turbid, and outcompete more specialist corals. To test the extent of their plasticity, we experimentally investigated light’s effect on growth, morphology, and symbiont community of *P. varians*. We fragmented four genotypes and grew clones in full sunlight, 2x layers, or 4x of 50% shade cloth. We calculated buoyant weight change, lateral growth rate, and colony rugosity (from 3D photomodels). We found all genotypes grew significantly faster in the lowest light and slowest in full sun. Colonies altered their morphology depending on light environment, forming more extensive and higher ridges in high light. We found significant differences in growth rate between genotypes. We used a metabarcoding approach to sequence the ITS2 region of Symbiodiniaceae in the coral tissue. We found no significant difference in Symbiodiniaceae community between treatment or genotype, and suggest coral host traits are more likely to explain genotype differences.

Seeing the Light: Evolution of Photosymbiosis in Marine Cockles

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Photosymbiotic associations between metazoan hosts and photosynthetic dinoflagellates are crucial to the trophic and structural integrity of marine ecosystems. However, long-term evolutionary dynamics of photosymbiosis in many marine metazoans are not well understood. Within Bivalvia, obligate photosymbiosis are found in two marine lineages: the well-known giant clams (Subfamily Tridacninae) and the heart cockles (Subfamily Fraginae). Both groups belong to the family Cardiidae and host symbionts from the same genus (Symbiodinium), although Fraginae also includes a lineage composed of several non-symbiotic species. To date, the phylogenetic relationship between the two subfamilies are not well resolved; and it is unclear whether the two lineages share a common photosymbiotic ancestor or evolved photosymbiosis independently. In this study, we established a backbone phylogeny for Cardiidae utilizing transcriptomic data. Multiple phylogenomic approaches were used to resolve the relationship between the two groups. This process was challenging due to conflicting gene signals and potential rapid divergence among the lineages. Overall, our results support a sister relationship between Tridacninae and Fraginae, that diverged around 78 million years ago. Although a sister relationship is recovered, ancestral state reconstruction still reveals two independent origins of photosymbiosis, one within Tridacninae, the other within the symbiotic Fraginae clade. Given the drastically different morphologies and adaptations to photosymbiosis between the two groups, this is not surprising. However, their newly revealed common ancestry brings a possibility that certain genetic/metabolic preadaptation existed in their common ancestor, which promoted both lineages to independently establish symbiotic relationships with *Symbiodinium* algae.

Cinachyrella as a model sponge genus for evolution, microbial symbiosis, and comparative genomics

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Our laboratory has been studying the sponge genus Cinachyrella for the past six years, as it is a common resident of Western Atlantic and Caribbean reefs, including the Florida Reef Tract. At least four, and perhaps up to six, *Cinachyrella* species (*Cinachyrella kuekenthali*, *C. alloclada* 1 & 2, *C. apion* and *C. arenosa*) occur or overlap in the Western Atlantic area. Several traits support the genus use as an experimental system: this sponge can be maintained for weeks and months in aquaculture, can reproduce via viviparous propagation or asexually (albeit at irregular times and unknown cues), and appears resistant to fouling. To date, a draft *Cinachyrella* transcriptome, metagenome and multiple microbiomes from various individuals have been sequenced. We have studied Cinachyrella spp. in the field and in laboratory experiments to investigate changes in holobiont physiology and microbial community structure in response to stressors such as crude oil and antibiotics. Electron microscopy of the holobiont ultrastructure has revealed both low and high microbial abundances in various *Cinachyrella* spp. For example, *Cinachyrella kuekenthali* and perhaps other congeners could be considered as high microbial abundance (HMA) sponges via TEM. A broader question regarding *Cinachyrella* spp. and other potential model sponges regards the specificity of their microbial symbiont communities (microbiomes), and their potential effects on holobiont divergence and speciation. For example, a recent study has shown that the presence of mitochondrial group I introns has divided sympatric Cinachyrella individuals into at least two *C. alloclada* species; there is also strong correlation of intron presence/absence with divergence in the microbiome composition of these same individuals. This finding suggests that host genetics (i.e. host divergence) can have a strong influence on microbiome structure, via currently unknown factors, even for visually identical, sympatric sponges which appear to have slight genetic differences.

Dicyemid mesozoan genome reveals adaptations to the parasitic lifestyle

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Parasitism has independently occurred more than 200 times across 15 animal phyla, yet remains a topic of debate how free-living ancestors evolved to parasitic organisms. Dicyemid mesozoans are microscopic endoparasites inhabiting the renal sacs of some cephalopods. They possess simplified body architecture without differentiated organs and have long fascinated biologists because of their incompletely known life cycles. Obtaining genomic data from enigmatic parasites would be essential to better comprehension of evolution of parasitism. Here I decoded the genome of *Dicyema japonicum* which is approximately 68 Mbp with substantially shortened introns. Comparisons of genomic data among bilaterians showed that *D. japonicum* retains fewer genes in most KEGG pathways, as in the case of four parasite species from different phyla that show a convergent gene number reduction in the metabolism pathways. In contrast, *D. japonicum* exhibits multi-copy gene clusters associated with endocytosis, perhaps reflecting its specialized nutrient-uptake strategy. Up-regulated transcripts at dispersal larvae stage indicate over-representation of gene ontology terms of motor activity and response to the stimulus. Dicyemids may utilize potential sensory functions to detect environmental cues in order to actively approach new hosts. In summary, the dicyemid genome provides a resource to uncover the molecular background of specialized physiologic processes and the mysterious life cycle of dicyemids, as well as for applying comparative genomic approaches to gain insights into the evolution of parasitism. Furthermore, genomes of parasites may adapt through eliminations of genes which are not necessary for parasitic lifestyle or through increasing gene copies corresponding to lineage-specific biological processes.

Comparative metatranscriptomes reveal the adaptive potential of coral holobionts under thermal stress

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Elevated sea surface temperatures pose a threat for coral reefs. Evaluating adaptive potential becomes increasingly important under the threat of climate change. Using a phylogentic framework, we have performed a controlled bleaching experiment on three different coral species. Through a comparative metatranscriptome analysis, we uncovered genes that have maintained conserved expression over evolutionary time that may have undergone expression level adaptation. Our analyses reveal both host and algal gene candidates with a potential adaptive expression involved in key metabolic functions such as protein processing, vesicle mediated transport, apoptosis, carbon concentration mechanisms, cell division and chlorophyll biosynthesis.

In addition, we observed that coexistent coral holobiont microbial associates display different responses and metabolic capabilities under high temperature stress. We find that each member has a unique response that can influence the holobiont's ability to cope with thermal stress. Thermotolerance may be explained the redundancy and the maintenance of key metabolic pathways from different microbial partners. These microbial functional contributions to coral holobionts can have conspicuous evolutionary and ecological outcomes under climate change.

Amplification of VWD domains preceded the origin of cypridinid luciferases and bioluminescence

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Bioluminescence is an ecologically impactful phenotype often used in communication including courtship signals whose origins increase rates of speciation. Because bioluminescence is strongly influenced by few or even single genes, learning how those genes originate is critical for understanding how genetic changes impact diversification. One origin of bioluminescence occurred in cypridinid ostracods (Crustacea), which employ complex courtship displays that differ among dozens of species from the Caribbean. Cypridinid bioluminescence involves *c-luciferase* enzymes, which contain only two deeply conserved sequences, both Von Willebrand Factor D (VWD) domains. Here, we characterize the history of VWDs from *c-luciferase*. We first analyzed VWDs in 10 arthropod genomes, finding them in parts of many different genes with distinct domain architectures. We next included VWDs from ostracod transcriptomes, discovering *c-luciferase* originated through novel fusion of distantly related VWDs. Unexpectedly, we found VWDs of *c-luciferase* to be part of a proliferation that predates the inferred origin of cypridinid bioluminescence. Although we still have much to learn about gene function, this mode of gene origin may be similar to Innovation Amplification Duplication (IAD), but with different timing. We find for ostracod *c-luciferase* that Amplification of VWDs began before functional Innovation and gene Duplication (AID). This order of events has implications for mechanisms of molecular evolution. Unlike IAD, which posits selection on function (bioluminescence) in all three stages, AID suggests molecular exaptation, whereby Amplification occurred for other reasons (perhaps still due to natural selection), before bioluminescence. These results illustrate how contingent, unpredictable genetic histories might contribute to ecologically impactful phenotypes.

Current "unknowns" in nematode genomics and biodiversity

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Nematodes are amongst the most diverse metazoans on earth, and often found in high abundances in both terrestrial and marine systems where they play key ecological roles. As parasites, they can infect a large number of hosts including invertebrate and plant species as well as humans. Despite this ubiquity, our knowledge of the diversity, ecology, and phylogeny of Nematoda is still limited to a few groups. For example, most of the nematode genomes available in public databases represent either a model organism (e.g. *Caenorhabditis elegans*) or a parasite (e.g. *Ascaris*, *Brugia*, etc. that infect humans). On the other hand, the greatest diversity of nematode species may be found in marine ecosystems, in particular poorly explored habitats such as the deep-sea. Although the first broad molecular phylogeny for the phylum Nematoda was published 20 years ago, with few exceptions, little progress has been made towards understanding the origin and diversification of this group. With the advance of sequencing technology (e.g. Illumina and other long-read technologies), molecular methods such as metabarcoding and metagenomics are becoming the gold standard in ecological-biodiversity surveys of small eukaryotes. These methods will not only improve our understanding on the diversity of these small groups, but also create opportunities to explore important aspects of their ecology and phylogeny. Herein, we provide an overview of the promise and challenge of –Omics studies focused on nematodes, and illustrate how new methods can contribute to improving our understanding of such a diverse group.

Expressed Exome Capture Sequencing (EECSEQ): A Method for Cost-Effective Exome Sequencing of Non-Model Organisms

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Exome capture is an effective tool for surveying the genome for loci under selection. However, traditional methods require annotated genomic resources. Here, we present a method for creating cDNA probes from expressed mRNA, which are then used to enrich and capture genomic DNA for exon regions. This approach, called “EecSeq,” eliminates the need for costly probe design and synthesis. We tested EecSeq in the eastern oyster, Crassostrea virginica, using a controlled exposure experiment. Four adult oysters were heat shocked at 36°C for 1 hr along with four control oysters kept at 14°C. Stranded mRNA libraries were prepared for two individuals from each treatment and pooled. Half of the combined library was used for probe synthesis, and half was sequenced to evaluate capture efficiency. Genomic DNA was extracted from all individuals, enriched via captured probes, and sequenced directly. We found that EecSeq had an average capture sensitivity of 86.8% across all known exons and had over 99.4% sensitivity for exons with detect- able levels of expression in the mRNA library. For all mapped reads, over 47.9% mapped to exons and 37.0% mapped to expressed targets, which is similar to previously published exon capture studies. EecSeq displayed relatively even coverage within exons (i.e., minor “edge effects”) and even coverage across exon GC content. We discovered 5,951 SNPs with a minimum average coverage of 80X, with 3,508 SNPs appearing in exonic regions. We show that EecSeq provides comparable, if not superior, specificity and capture efficiency compared to costly, traditional methods.

Draft genome of Bryozoan *Bugula neritina* – a colonial animal packing powerful symbionts and potential medicines

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Many animal phyla have no representatives within the catalog of whole metazoan genome sequences. This dataset fills in one gap with a complete genome of *Bugula neritina* (phylum Bryozoa). This species stands prominently outside ecology and into biomedical realms due to its production of potentially therapeutic bryostatins.
Here we present a draft assembly of the *B.neritina* genome obtained from PacBio and Illumina HiSeq data, as well as genes and proteins predicted de novo and verified using transcriptome data. The genome is estimated at 232.5 Mb based on 24x coverage PacBio reads and 154x Illumina reads. We do exercise caution with this estimate since the distribution of 23-mers have indicated a noticeable level of heterozygosity. Overall the *B.neritina* genome displayed a low to moderate repetitive DNA content - 0.94 % SINEs, 2.59 % LINEs, 6.46 % LTRs, 3.33 % DNA elements, 20.58 % interspersed and 7.25 % unclassified repeats. We predicted 24,814 genes, of which 96.5% (23,947) were functionally annotated. This genome fills in a gap in the sequencing of animal genomes for understanding the tree of life. These sequences will permit a better understanding of host-symbiont interactions at the genomic level, and also contribute additional phylogenomic markers to evaluate Lophophorate or Lophotrochozoa phylogenetics relationships. The effort also fits well with plans to ultimately sequence all orders of the Metazoa.

A hybrid-hierarchical genome assembly strategy to sequence the invasive golden mussel, *Limnoperna fortunei*

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Background: For more than 25 years, the golden mussel, *Limnoperna fortunei*, has aggressively invaded South American freshwaters, having travelled more than 5000 km upstream across 5 countries. Along the way, the golden mussel has outcompeted native species and economically harmed aquaculture, hydroelectric powers, and ship transit. We have sequenced the complete genome of the golden mussel to understand the molecular basis of its invasiveness and search for ways to control it.
Findings: We assembled the 1.6-Gb genome into 20 548 scaffolds with an N50 length of 312 Kb using a hybrid and hierarchical assembly strategy from short and long DNA reads and transcriptomes. A total of 60 717 coding genes were inferred from a customized transcriptome-trained AUGUSTUS run. We also compared predicted protein sets with those of complete molluscan genomes, revealing an exacerbation of protein-binding domains in *L. fortunei*.
Conclusions: We built one of the best bivalve genome assemblies available using a cost-effective approach using Illumina paired-end, mate-paired, and PacBio long reads. We expect that the continuous and careful annotation of *L. fortunei*'s genome will contribute to the investigation of bivalve genetics, evolution, and invasiveness, as well as to the development of biotechnological tools for aquatic pest control.

Delineation of the Caribbean fire corals (*Millepora* spp.) using transcriptomic data

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Millepora is a relative rich-species genus of hydrocorals, with 18 species distributed around the globe. It is considered one of the important reef building cnidarians. The current diversity of the Caribbean *Millepora* species consists of *Millepora complanata*, *M. alcicornis*, *M. squarrosa* and *M. striata*. Here, we report the *de novo* transcriptome assembly and phylotrascriptomic analysis of *M. alcicornis, M. complanata, M. squarrosa* and a new ecomorph (*Millepora* sp.) found in exposed *Thalassia* beds and mangrove areas in southwest Puerto Rico. Over 345 million reads were obtained for the analysis of the transcriptomes (Illumina HiSeq4000; 2x150bp). The analysis pipeline consisted of assembly with Trinity, BUSCO, RSEM, and ORFs calling for each transcriptome, followed by ontology (Blast2GO) and phylogenetic analysis. The phylogenetic analysis was performed using distinct custom bash programs to select homologous sequences among the transcriptomes, resulting in 10,797 homologous sequences. Concatenation analysis (with either Maximum Likelihood or Bayesian inference) and a coalescence-based analysis were performed to the dataset. Concatenation results presented a topology supporting a clade of *M. complanata* and *M. alcicornis*, with *Millepora* sp. outside the clade and *M. squarrosa* as outgroup. The coalescence-based tree estimation analysis (ASTRAL-II), presented a different topology resulting, with *M. alcicornis* forming a clade with *Millepora* sp. rather than with *M. complanata*. Our coalescence analysis indicated that there is a very high degree of incomplete lineage sorting, suggesting a very recent time of divergence among three out of the four Caribbean *Millepora* species.

Development of Open-Source Tools for Comparative Genomics Analyses: Discrimination of Technical vs Biological Variation in Genes and Genomes of Invertebrate Higher Taxa from NCBI GenBank

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Genome sequencing and other -omics scale data from diverse invertebrate taxa are increasingly available in public databases, including in NCBI's GenBank Assembly, Genome, and Protein databases. The GenBank umbrella includes Gene, RefSeq, and Taxonomy databases, with linkages between nodes in other internal NCBI databases. Such linked data can be retrieved using the NCBI E-utils set of API tools; we use these here for gene-, reference genome-, and taxa-delimited comparative analyses of invertebrate genomic data. We developed a set of open-source scripts, Linux command-line and R tools called GeneFamTaxScan (https://github.com/PhyloGrok/GeneFamTaxScan) to perform statistical comparisons of invertebrate Genome- and Gene- specific database content to better understand biological and technical variation in the data. *Biological* parameters include genomic DNA, mRNA and ncRNA sequences, and inferred peptides. *Technical* parameters include genome completeness (contigs, scaffolds, chromosomes) as QC metrics. Technical and biological genomic data was retrieved, and statistical comparisons made between invertebrate species and higher taxa to gain biological insight.
Results from the following GeneFamTaxScan analyses are presented:
1) Comparison of Genome/Assembly/Contig-level QC data such as sequence length and contig number between Porifera, Cnidaria, Mollusca, Annelida, Lophotrochozoa, Platyhelminthes, Nematoda, Chelicerata, Mandibulata, and Echinodarmata. Curated NCBI RefSeq Genomes were used for reference, taxa-associated means comparisons and regression analyses were performed, and technical outliers for assembly- and contig-level accessions were identified and modelled.
2) Comparison of Gene-centric data for deeply conserved genes of the microRNA biogenesis pathway was performed. Average sequence length, regional synteny, and other data was used to discriminate between putative misassembly/misannotation and evolutionary divergence.

The epigenetic landscape in the bdelloid rotifer *Adineta*

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The epigenome is formed collectively by a set of mechanisms that have the capacity to direct regional and local activation or silencing of genes, transposons, and other regions in the genome. These mechanisms include covalent modification of histones, DNA methylation, and non-coding RNA pathways. The genome contributes to epigenetic processes by encoding the necessary enzymatic machinery, such as methyltransferases, demethylases, or RNA-mediated silencing proteins (Dicers, Argonaute-Piwi, RNA-dependent RNA polymerase).

Rotifers of the class Bdelloidea are microscopic freshwater invertebrates able to survive desiccation at any life stage and to reproduce asexually. The genome of the bdelloid rotifer *Adineta vaga* is unusual in having over 8% of its genes originated from non-metazoan (predominantly bacterial) sources. Nevertheless, transposable elements (TEs) span only about 3.5% of the *A. vaga* genome. We investigated genome-wide epigenetic landscapes in two *Adineta* spp. (the *A. vaga* reference strain and the *Adineta* sp.11-2 natural isolate), including pi-like small RNAs, DNA methylation and histone modifications. The correlations between TEs, mRNA, piRNA and host genes confirm the expected role of piRNAs as central players in transposon silencing and horizontally acquired foreign genes.

In methylation, *Adineta* spp. display a unique and highly unusual combination of epigenetic marks, the presence of N6-methyladenine (6mA) and N4-methylcytosine (m4C) base modifications. Further, we investigated genome-wide distribution of three histone marks (H3K4me3, H3K9me3 and H3K27me3). Finally, we generated a genome-wide map of epigenetic modifications and revealed correlations between different epigenetic marks in order to shed light on molecular mechanisms underlying epigenetic regulation in these bdelloid rotifers.

Improvements in phylogenetic models for estimating deep evolutionary divergences

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Genomic and transcriptomic data from diverse lineages of organisms across the tree of life have allowed us, for the first time, to address major outstanding questions regarding the 'deep' structure of the tree of eukaryotes. At the same time, we have become aware of the limitations of our phylogenetic models of sequence change to accurately capture the molecular evolutionary process on the billion-year time scale, especially the complexity of the process across sites, genes and taxa. Although failure to correctly model these complexities can lead to serious phylogenetic artifacts there are major debates over which of these aspects of the process are most important to model. Here I will describe new approaches for modeling heterogeneity in the substitution process across sites in the maximum likelihood framework and show how these methods perform in simulation and real phylogenetic problems. I will also compare the relative importance of modeling site-heterogeneity versus variation in rates across proteins in large multi-protein analyses for accurate deep phylogenetic inference.

Abundant DNA Methylation in the demosponge *Amphimedon queenslandica* is involved in genomic evolution and transcriptional regulation

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DNA methylation is an epigenetic mechanism with roles that go from the fine tuning of transcription to genome wide dynamic *acclimation* to changing environments. It is mostly found as 5-methyl Cytosines (5mC) present at least in a small fraction in most of studied metazoans. While recent work have confirmed the presence and regulatory functions of DNA methylation in non-bilaterians, its role and distribution in Porifera has never been addressed. In this study, we performed whole genome bisulfite sequencing in the demosponge *Amphimedon queenslandica* and show that DNA methylation occurs mostly in Cytosines of CpG dinucleotides that concentrate in coding regions. While high levels of gene-body methylation are positively correlated with higher expression and cooccur with the histone modification H3K36me3, they do not contribute to the amelioration of spurious transcription as described in other metazoans. Nonetheless, per-exon methylation levels are predictive for exon retetion suggesting a role in regulation mRNA splicing. Additionaly, composition analyses of *Amphimedon* and other sponge genomes consistently revealed a unique bias of their dinucleotide abundances. A strikingly low amount of CpGs and a corresponding enrichment for TpG and CpA suggest a long history of deamination of methylated CpGs. Interestingly, this bias is significantly higher in introns than in exons and exhibits a stronger association to methylation than to codon usage. These results speak in favour of DNA methylation as a component of early metazoans regulome and its abundance supports is role as the main cause of the noteworthy footprint in poriferan genomes also described in this study.

Differential gene expression data elucidate systemic effects of Wnt signaling inhibition during segmentation in the spider *Parasteatoda tepidariorum*

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Segmentation is a key characteristic of arthropods linked to the evolutionary success of this lineage. The formation of segments, both along the antero-posterior body axis and the proximo-distal appendage axis, requires the activity of the Wnt family of secreted proteins, as inferred from functional data in model organisms (*e.g. Drosophila melanogaster*). Comparable data are limited in lineages like Chelicerata (e.g., spiders and mites), the sister group to the rest of the arthropods. Here we examined the inhibition of canonical Wnt signaling in the cobweb spider *Parasteatoda tepidariorum* using parental RNA interference (pRNAi) against the Wnt-1 co-receptor *arrow* (*arr*; vertebrate homolog: *LRP5* and *LRP6*), a key member of the canonical Wnt-signaling pathway in holometabolous insects and vertebrates. Toward a more refined characterization of the phenotype, we sequenced the transcriptomes of embryos displaying loss-of-function phenotypes, referencing negative control counterparts at two key stages of development, and mapped reads to the recently sequenced cobweb spider genome. We show that knockdown of *Ptep-arr* resulted in reduced expression of almost all Wnts, various segmentation genes (e.g., *Notch*, *Delta*), and posterior Hox genes patterning the body region formed by sequential segment addition (the opisthosoma). Intriguingly, differential gene expression (DGE) analysis and in situ hybridization revealed that *Ptep-arr* phenocopies overexpress *Wnt8* and its target *caudal* (*cad*). Our results elucidate a complex role for canonical Wnt signaling in regulating the anterior segment addition zone via Notch-Delta signaling and modulation of the Wnt8-cad circuit. More broadly, this work underscores the diagnostic power of DGE tools in categorizing catastrophic phenotypes.

The contribution of a diverse set of metazoan draft genomes and improvement of metagenomic analyses of meiofaunal communities

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Meiofauna (benthic metazoans < 1mm) encompass an extremely broad and diverse set of creatures. They are important contributors to ecosystem functioning through their roles in nutrient cycling, sediment stability, and food web interactions. Shifts in meiofaunal biodiversity may reflect environmental changes, which can be revealed almost instantly thanks to the organisms' short generation time and low dispersal capability. Therefore, surveying these communities is fundamental to the assessment of natural and anthropogenic stresses, restoration, pollution monitoring, and ecosystem health. Current molecular studies typically employ metagenomic sequence analyses, but are very limited due to a historically low representation of meiofaunal genomic data in public repositories and the lack of adequate genetic markers to capture population level changes. Here we report on an expanded set of reference genomes and discuss novel bioinformatics workflows to investigate meiofaunal communities. These new genomes encompass 160 individuals representing 77 unique taxa and primarily focused on Annelida, Nemertea, Platyhelminthes, Gastrotricha, and Nematoda. To test the influence of these new resources on metagenomic studies we conducted a comparative analysis with both mock meiofaunal communities and environmental sediment samples. We demonstrate that the expanded reference genomes improve the accuracy and breadth of sequence identification from metagenomic datasets. Furthermore, by utilizing a collection of 978 universal orthologous and a robust reference tree we place novel environmental sequences directly into phylogenetic perspective. These results allow us to evaluate the progress towards a more detailed description of meiofauna communities.

Whole genome resequencing reveals genomic basis of local adaptation to extreme environments in the ascidian *Phallusia nigra*

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Invasive species are emerging models for the study of rapid local adaptation due to their capacity to colonize new and distinct environments. *Phallusia nigra* is a widespread invasive species whose geographic distribution extends into the Persian/Arabian Gulf (PAG), a young sea that experiences mean summer temperatures of ~34□C and salinities of 40-45psu. Furthermore, in mangrove environments within the southern PAG, *P. nigra* is exposed to temperatures >37□C and salinities exceeding 50psu. Consequently, PAG *P. nigra* populations represent an ideal opportunity to study rapid local adaptation of an organism living at the upper limits of its environmental tolerances. Here, we investigate local adaptation to different environments along the coast of the southern PAG using whole genome sequencing of individuals from highly variable mangrove sites (daily range < 10□C) and comparatively more stable reef environments (daily range ~1-2□C). First, we produced a draft assembly of the *P. nigra* genome, which displays high heterozygosity consistent with other ascidian genomes. We found weak population structure between all sites in our study (all pairwise FSTs < 0.01), however, outlier tests identified regions of the *P. nigra* genome that are consistently differentiated between replicate mangrove and neighboring reef populations. Candidate genomic regions under selection include genes involved in proteolysis, as well as genes associated with hypersalinity adaptation in other invertebrates. These results highlight the mechanisms involved in rapid adaptation to extreme environments, the maintenance of adaptive polymorphisms despite high gene flow, and emphasize the utility of whole genome sequencing of invasive species for characterizing adaptive processes.

Development of cellular assays for characterization of coral cells using fluorescence-activated cell sorting (FACS)

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The future of coral reefs and the biodiversity in these economically-critical ecosystems is threatened by climate change and other human induced stressors. Currently, there are multiple coral genomes, over twenty coral transcriptomes, many microbiome studies, and an extensive literature of the symbiotic relationship between *Symbiodinium* and corals. However, little is understood about the diversity of coral cells, their functions, and the cell-specific microbial partnerships with viruses and bacteria. To address this gap, we have used fluorescence-activated cell sorting (FACS) to isolate coral cells based on universal cell markers. While many universal markers are unable to distinguish different cell types, we have successfully isolated unique cell populations using several vital cell markers, including those designed for reactive oxygen species (ROS), acidic organelles such as lysosomes, phagocytosis via uptake of fluorescent microspheres, and aldehyde dehydrogenase. These four unique markers provide for an intriguing panel to study immune and stem cell function, neither of which are yet greatly understood in corals. In addition to developing these non-specific assays to separate out coral cells, we are utilizing RNA-Seq and 16S rRNA amplicon sequencing to examine the variation in gene expression and background microbial communities in these different cell populations. Understanding the role of these various cell types will lead to a better understanding of how different cells respond to environmental stress, which could greatly aid in restoration and conservation efforts.

New insights on oyster genomics: the flat oyster as a model for disease and environmental adaption studies

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In Europe, the flat oyster *Ostrea edulis* aquaculture remain limited due to the high level of mortality associated with the two parasites *Bonamia ostreae* and *Marteilia refringens*. To improve the O. edulis production and allow the restauration of natural populations, a European research program called PERLE 2 has been promoted based on the development of molecular tools to select resistant and susceptible individuals. Individuals issued from natural populations have been used to produce bi-parental families in hatcheries that will be settled in different oyster production locations to test their susceptibility to various environmental parameters including parasites but also temperature, salinity and level of immersion. Selected resistant and susceptible families will be then genotyped using RADseq approach to develop sets of genetic markers (SNPs) associated with either parasite resistance and/or susceptibility, additional fitness traits (growth, survival,…) and environmental parameters. In this program, both oyster and parasites genome will be sequenced using a combination of NGS technology. Several additional analyses will be conducted including a comparative genomic approach between other oyster species and between several flat oyster populations. We will present the genomic data that have been already produced including transcriptomic and genomic analyses in a comparative study with other oyster species.

PhyloPyPruner: Contamination-Aware Tree-Based Orthology Inference

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Large-scale phylogenetic analyses rely on orthology inference methods to curate sets of sequences related by speciation (i.e., orthologs) rather than gene duplication (i.e., paralogs). Graph-based “orthology” inference methods typically cluster sequences together based on an all-vs-all BLAST followed by Markov clustering, but the output of such approaches often contains paralogous sequences. In tree-based methods, sequences grouped together using a graph-based method are used to build alignments and trees. Trees are then trimmed down to subtrees of orthologs where each taxon is represented by no more than one sequence per gene or, if there are multiple sequences from one taxon, those sequences form a clade (i.e., they are splice variants or inparalogs). Unfortunately, contaminant sequences present in even a single taxon can result in such approaches erroneously discarding large subtrees worth of sequences. PhyloPyPruner is a novel program for tree-based orthology inference that employs diverse methods for identifying and excluding paralogy and contamination. PhyloPyPruner implements previously published paralogy pruning algorithms, outgroup and midpoint rooting, a refined algorithm for monophyly masking, and the ability to work with and collapse weakly supported nodes into polytomies. Paralogy frequency (PF) measures how frequently a taxon has sequences inferred to be paralogs. Taxa that display a high PF, which might indicate contamination, may be excluded from the analysis. Taxon jackknifing excludes taxa one by one and provides statistics of the output alignments in each case. In addition, taxonomic groups can be defined to keep track of how frequently taxa are placed within the group to which they belong. Contaminants can then be identified and excluded, by finding taxa that continuously fall outside their expected group.

Phylogenomics of class Anthozoa (Cnidaria) using universal target-enrichment baits

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The anthozoan cnidarians (e.g., corals, sea anemones) are an ecologically important and diverse group of marine metazoans that occur from shallow to deep waters worldwide, and include some of the ocean's most important ecosystem engineers. Our understanding of the evolutionary relationships among the ~7500 species within this class is, however, deeply flawed. Molecular phylogenetic studies have revealed widespread homoplasy in morphological characters and widespread polyphyly at the ordinal, family, and genus levels. Resolution of both deep and shallow nodes in the anthozoan phylogeny has been hindered by a lack of phylogenetically informative markers that can be sequenced reliably across taxa whose divergence may pre-date the Cambrian. While recent phylogenomic analyses have supported the reciprocal monophyly of sub-classes Octocorallia and Hexacorallia, resolution of the ordinal relationships within each clade requires more comprehensive taxon-sampling than can be achieved with transcriptomic approaches. Using available anthozoan genomes and transcriptomes, we designed a set of 16,306 target-capture baits for enriching both ultraconserved elements (720 loci) and exons (1071 loci). Target enrichment was conducted on 242 anthozoans, representing all orders and a majority of families. Illumina sequencing of enriched genomes recovered 1774 loci, with a mean of 889 ± 253 loci per species. Maximum likelihood analyses of a 925-locus dataset with 50% taxon occupancy yielded highly resolved trees. In addition to answering long-standing questions about evolutionary relationships at the ordinal and family level, preliminary data for 69 *Acropora* spp. suggest that this approach will also facilitate delimitation of species, offering a more versatile alternative to RAD-tag methods.

The genome of the chiton *Acanthopleura granulata*

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Chitons (Polyplacophora) are a group of intertidal molluscs that graze on algae by scraping rocks with their tooth-bearing radula. Feeding is facilitated by teeth coated with iron (magnetite), offering incredible abrasion resistance without compromising flexibility. To better understand the mechanisms of iron deposition, we sequenced a draft genome of *Acanthopleura granulata*, the west Indian fuzzy chiton. A single individual was collected from the Florida Keys and DNA extracted from foot tissue. One lane of an Illumina HiSeqX (2 x 150bp PE) was combined with 4 flow cells of Oxford Nanopore GridION data, producing high coverage at relatively lower cost. Over the course of nanopore sequencing, the release of new kit chemistries permitted examination of developing third-generation sequencing on a non-model organism. Comparisons were made between multiple nanopore base-calling and adapter-trimming softwares, and between several different assembly programs. Bionano SAPHYR optical mapping will be added in the future to produce a higher-quality assembly. Genomic data will be combined with differential expression analyses across the regions of the radula, highlighting genes relevant to the iron mineralization process. Our chiton genome will join fewer than 10 sequenced molluscan genomes, and is the first genome of clade Aculifera (chitons and aplacophoran, the sister group to all other molluscs). This data will thus inform subsequent studies of iron mineralization as well as molluscan relationships and evolution.

Accessing to the miRNA complement in tunicate genomes, an *in silico* approach

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Due to the significant role of microRNAs on regulation, this tiny RNAs have been identified nowdays as acting as very important players on key developmental processes and into an evolutionary context, the emergence of new miRNA families in metazoan has been correlated with morphological innovations in animals, such as the case of chordates, nevertheless, the genomic distribution of loci and its genomic context are still poorly understood in non-model marine organisms albeit is particularly consider conserved throughout plants or in vertebrates with some particular excepcions due to the dinamics of the origin of many families clade-specific.

In marine chordates, current effort to annotate miRNAs is still very vertebrate-based due to the of huge amount of genomic information available and is more dramatically limited by the small miRNA expression experimental data of invertebrate organisms. So in in this survey, we focus on the study of the genome organization of miRNAs repertoire in the chordate group Tunicate and other chordates to get annotations more realiables and clade-specific.

For that, genomes of sixteen tunicates, two cephalochordates and three vertebrates were screened by homology searches, in first place, based on six modified blastn strategies centered on the current miRNAs annotation of eleven bilaterian species and in a second homology search based on Hidden Markov Models (HMM) of miRNAs. Outputs were validated crossing structural alignments against the metazoan-specific Covariance Models (CM), to finally obtain a select set of candidates located over non-redundant subgenomic regions of loci candidates of miRNAs.

Our results revealed that was most frequently found the *mir-10* family in Olfactores, in contrast to cephalochordates were the family *mir-233* is predominant. Finally to decipher the genome organization of miRNAs loci, a clustering strategy was implemented centered on search of uninterrupted contiguous positions of loci and other genomic elements. Finally, we found about that 14% of miRNAs loci in tunicates are located inside a cluster but their cluster size is reduced in comparison to other chordates.

A phylogenomic framework for Decapoda

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Comprising over 14,000 living species, decapods (crabs, shrimp, and lobsters) are the most instantly recognizable crustaceans, representing a considerable global food source. Although decapod systematics have received much study, limitations of morphological and Sanger sequence data have yet to produce a consensus for higher-level relationships. Here we introduce a new anchored hybrid enrichment kit for decapod phylogenetics designed from genomic and transcriptomic sequences that we used to capture high-throughput sequence data from 94 species, including 58 of 179 extant decapod families, and 11 of 12 major lineages. The enrichment kit yields 410 loci (>86,000 bp) conserved across all lineages of Decapoda, eight times more molecular data than any prior study. Phylogenomic analyses recover a robust decapod tree of life strongly supporting the monophyly of all infraorders, and monophyly of each of the reptant, ‘lobster’, and ‘crab’ groups, with some results supporting pleocyemate monophyly and some supporting mud shrimp paraphyly. New insights into decapod relationships provide a phylogenomic window into the evolution of morphology and development, and a basis to rapidly and cheaply expand taxon sampling in this economically and ecologically significant invertebrate clade.

Monaco Explorations – Climate Change Impacts on Ocean and Human Health

Didier Zoccola

Centre Scientifique de Monaco

Within the framework of Monaco Explorations, four CSM teams will investigate the links between human and ocean health under the influence of climate change and local disturbances. We will focus on two major themes:

1/ *comparative response of tropical corals to human-induced perturbations in shallow and mesophotic reefs*

While coral reefs are in decline due to global change as well as more local impacts, mesophotic coral ecosystems (MCEs) have been spared from the deterioration and may act in the future as a refugee for shallow-water organisms. It is why we will study the physiology, ecology, and microbiome of key coral species found in both shallow and mesophotic environments. Meta-transcriptomics and meta-genomics will be performed to analyse the entire holobiont. Furthermore, genome sequencing of two key coral species will be done. Using different bar-coding sequences, the taxonomy of mesophotic coral species will also be investigated. At last, to assess whether deep reefs under human pressure are more threatened, pristine and polluted areas will be compared.

2/ *The Role of Global Climate Change in the Emergence of potential Human Pathogens from the Ocean*

Due to the increase of water temperature, marine microbial communities are changing and these modifications can have serious consequences on human health, supported by recent correlations between worldwide microbial population trends and human health problems. Within the scope of Monaco Explorations, we propose to study the diversity of potentially pathogenic microorganisms along a depth gradient and to investigate the effect of climate change on the expression of virulence factors.