

ASCIDIAN NEWS*

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Many many thanks to the large number of AN readers who sent in contributions and for letting me know how important AN continues to be! There are **87** new publications listed at the end of this newsletter. Please keep in touch and continue to send me contributions for the next issue!

***Ascidian News is not part of the scientific literature and should not be cited as such.**

NEWS AND VIEWS

1. From Ayelet Voskoboynik (ayeletv@stanford.edu): We are excited to announce that the next **Intl. Tunicata Meeting (ITM 2024)** will be held in California in July 2024. In order to keep costs reasonable, we are planning to hold this event at Univ. of Calif. Santa Cruz. We have requested housing and facilities from **July 22nd to July 26th, 2024**. Although they will only be able to confirm the dates in September, we wanted to share these tentative dates with you. More information regarding registration, abstract submission, and other details will be provided in September.

2. The 11th Intl. Conference on Marine Bioinvasions was held 15-19 May 2023 in Baltimore, Maryland, after having been postponed from last year due to covid. There were a large number of enthusiastic participants (including your AN editor) and many of the talks and posters included ascidians. You can find the complete program at: <https://marinebioinvasions.info/program>. The titles are nested within each session so click on the session to view the titles, then click on each title to read the abstract. The official book of abstracts will be released in late June on the conference website.

Two special issues will be published to highlight research presented at the conference: one in [Aquatic Invasions](#) focusing on academic research in bioinvasions, one in [Management of Biological Invasions](#) focusing on applied research on invasive species. All ICMB XI participants are encouraged to submit their research manuscripts for publication in the conference proceedings. Please see the conference website for details. **Manuscript submission deadline: November 1, 2023.**

3. Anna Di Gregorio, New York Univ. (adg13@nyu.edu) and **Marie Nydam** (mnydam@soka.edu) organized an online **International Symposium on Women in Tunicate Biology** on March 28-29, via Zoom. Scientists from across the globe met to celebrate the contribution of women to research on different aspects of tunicate biology. All of the major fields of tunicate biology were represented, including developmental and stem cell biology, regeneration biology, ecology and taxonomy. During the first day, the participants honored women scientists who had either passed away or retired. The second day was dedicated to scientific presentations on ongoing research in the field.

The proceedings of the symposium will be published in a Special Issue in *genesis: The Journal of Genetics and Development*, which will include a meeting report as well as individual contributions dedicated to the scientists who were celebrated. These contributions will be published in letter format

and will contain reproduction of drawings and figures that encapsulate each scientist's research focus. We are inviting submissions of short summaries of each scientific presentation, also in letter format. A tentative deadline of **June 15** has been set. Please see the website:

<https://onlinelibrary.wiley.com/page/journal/1526968x/homepage/women-tunicate-biology>

This special Issue is expected to become a resource for all scientists interested in tunicate biology, a reference for early and contemporary work in this field of science, and an inspiration for all women scientists. If you were not able to participate in the symposium and you want to share information on a mentor or an inspiring woman whose work on tunicate biology has inspired you, please contact us! Thank you, Anna Di Gregorio and Marie Nydam.

4. An important message from Laure Corbari on behalf of the Paris Museum (MNHN, Muséum National d'Histoire Naturelle, Paris) staff: We would like to share with you our concerns about the project of relocation of our collections. The MNHN administration just decided to choose the city of Dijon (300 km away from Paris) instead of Ris-Orangis (Suburb of Paris). We are shocked by this decision which implies a separation of the research and collection management teams. We have launched an online petition : <https://chnq.it/sKR4pLZSdJ> . Be sure to sign the petition, it is even more important if it comes from taxonomists living abroad.

Please help save the MNHN collections: Here is an open letter disclaiming the project of relocation of marine invertebrates and vertebrates collections of Paris Museum. Over two centuries old, the National Museum of Natural History (MNHN) of Paris, had always been inseparable from its collections. These collections are considered as an important research instrument dedicated to the understanding the Nature. These archives of the Nature are enriched by field-explorations all over the world with the awareness that this inventory is far from to be completed. The collections are also central in the comparative method that scientists apply for proposing new taxonomic hypotheses and questioning them with new collections or new analysis tools. As we know, the rate of species extinction is faster than the time needed by scientists to describe them: Enriching, preserving these collections and enhancing their value are major challenges for science and society.

These scientific collections are the true heart of the Paris Museum. Their management, growth and improvement rely on the multiple activities performed by MNHN staff. The MNHN teams use them through research, dissemination, teaching and expertise activities. The collections are unique in the world, due to their history, diversity, quality, and status as a reference for the scientific community, but also due to the current growth dynamic generated by the scientific expeditions led by the MNHN research teams for exploring the world. These dynamic and recognition are illustrated by many metrics: about 1100 scientific publications/year relying on these collections, in 2022 a total of 2752 requests for access to the collections, the scientific importance of the reference material hosted in MNHN collections (type material), or even the fact that over the last 10 years 23% of all the new marine species discovered in the world have been described from MNHN material. Despite this international recognition, scientific visitors who had the chance to visit these collections located to the Jardin des plantes, are struck by the damaged rooms, the poor working conditions of the management and research teams, and by the lack of financial and human resources available. Yes, there is an urgent need for more space, for renovation and improvement of the working conditions, which are far from the standards that should prevail in the 21st century.

Originated from the protocols of the Convention on Biological Diversity, the questions of data accessibility of these new collections, as well as the sharing of the scientific knowledge, are major issues for the teams that sample, manage and ensure the valorisation of these collections. For more than fifteen years, the funds raised by these teams, as part of the funding dedicated to Research, have partially compensated the resources lacking to the collections. Our teams have developed ambitious research programmes in taxonomy, based on the growth and enhancement of collections, but also on the data traceability from the field to the scientific publications. These projects, funded by the French Research Agency (ANR), or European Research Council (ERC), French conservation

authorities but also private foundations and sponsors, allowed to hire technical staff for achieving the challenges of collection data accessibility and open science for disseminating biodiversity knowledge.

At the end of 2021, the MNHN administration put forward an expectation from Paris authorities, which seemed no longer to want large quantities of ethanol to be stored in the heart of Paris. MNHN initiated then a process of collections relocation by opening a call for a new place outside Paris. This project concerns all the collections in ethanol, as well as the dry collections and associated library: all the marine invertebrates and part of the vertebrates collections are concerned. This call targeted local and regional authorities, able to propose field and resources to set up a new building for these collections. A major element was the requested distance from the historical Museum: ideally less than 2 hours from the Jardin des Plantes. We just learned from the newspapers (Le Parisien, April 17th) that the MNHN administration has chosen the city of Dijon (300 Km from Paris) over the city of Ris-Orangis (Suburb of Paris), after an evaluation of the 39 cities that have submitted a proposition. Two decisions - moving the collections outside Paris and choosing the city of Dijon to host them - were taken without concertation and are going to be validated soon.

At mid-process, we, the collections management and scientific teams who work daily on these collections, have been consulted. However, “consulting” does not mean “listening”. To our questions about the impossibility of finding a solution to keep the collections closer to Paris (suburbs), the answer was simply “not possible”. Our main concerns are about the time and cost of travels to Dijon. The answer: We have to adjust and organise our schedule accordingly, without specifying who is paying for the travel costs, or how these travel times would be considered. Yet the decarbonisation of research is one of the major projects put forward by MNHN and French instances: why this choice of a distant place involving numerous trips for people and collections? Another response by the MNHN administration is that virtual specimen (digitisation) will allow remote consultation. But the specimens of collections are not books whose digital version provides remote access to its content. This misunderstanding of taxonomy is astounding because working on collections requires direct access to specimens for observing, comparing and acquiring new data. Time is needed for describing them, clipping tissues, analysing them with modern scientific tools and for generating new knowledge. We are disappointed by this backward-looking understanding of collections, as archives of past research, which could be stored in warehouses, without consultation: Collections are the raw material of actual research, and an open window on the future of biodiversity.

Every day, we, the collection management and research teams, are working on the collections, whether to verify information on a label, to study specimens to clarify an identification, to take a sample, to take a photograph, to answer to requests from our colleagues, etc. What about the hundreds of scientific visitors who request access to these collections each year? How will we maintain this activity at 300 km distance? The backbone link between the technical teams (relocated to Dijon) and the research teams (on the Jardin des Plantes) will be definitely broken! How will the staff and students be trained if the researchers are in Paris and the collections in Dijon? How can we justify the funding of research projects on the collections if they are not accessible to the teams every day? Thinking that the current activities and dynamics will be maintained 300 km away is illusory. We are the first generation of scientists to be simultaneously aware that we only know a quarter of the planet's species, and that half of them could disappear by the end of the century. In this context, how could we imagine to break the collections-research link? The main museums of the world develop networking with the common objective of inventorying biodiversity, making it accessible in order to better understand it and better preserve it: what message will the Paris Museum send to the international community with this project? We all recognise the need for more space, the need to improve the conditions of work for the technical and research teams. But all, we are also equally opposed to this project of relocation of the collections to Dijon: the solution would be much worse than the problem.

We therefore call for the cancellation of this project and request the launch of a new process involving discussion with the staff. A process based on a common reflection with the concerned

teams is the only way to bring out new propositions that have to be validated by a democratic vote.

- First action: an online petition <https://chnng.it/sKR4pLZSdJ>
- An email address to share your ideas for action: sauvonslescollections@gmail.com

5. An Ascidian Taxonomy Workshop was held **June 5-9, 2023** in southern California at Soka University of America, Aliso Viejo, CA, USA. Hosted by Marie Nydam (mnydam@soka.edu), Susanna López-Legentil (lopezlegentils@uncw.edu), and Lauren Stefaniak (lstefania@coastal.edu); funded by NSF DEB RUI #2122475. The workshop focused on living specimens collected at local marinas. There were lectures on ascidian phylogenetics, taxonomy, identification and ecology, as well as extensive hands-on practice with dissection and identification of living specimens.

6. From Takahito Nishikata: We are organizing a special issue on "**Ascidian Early Development**" that we hope you will be interested in contributing to. The International Journal of Molecular Sciences has become a popular journal in the field with a steadily increasing impact factor (the latest IF 6.208) https://www.mdpi.com/journal/ijms/special_issues/2E36TWWV9PD. Please contact us, or the Assistant Editor, Jenine Wang (jenine.wang@mdpi.com) for further information, and please forward this message to any colleague who you think may be interested in contributing. **15% discount** would be offered for your submission, in which you act as the only correspondence author, if it is submitted before **30th Sept. 2023**. If you are interested in contributing, please let us know – a tentative title and a very short summary of 1-2 sentences will do.

You can reply directly to Jenine Wang. Then we will make a record and follow up submission details with you.

Yes: We can submit by 30th Sep 2023 and accept the discount.

Yes: We need more time to finish the paper. The expected submission date is XX XX XXXX.

No: We have no papers.

IJMS has a rigorous peer-reviewing process for all manuscripts, but is proud to provide the first decision to authors approximately 15.7 days after submission. Upon acceptance the paper is rapidly published online.

Prof. Dr. **Takahito Nishikata** (nisikata@konan-u.ac.jp), Konan University Research Institute for Human Health Science (RIH2S), Konan University; Prof. Dr. **Takeo Horie** (horie.takeo.fbs@osaka-u.ac.jp), Osaka University Graduate School of Frontier Biosciences.

7. From Simon Blanchoud (simon.blanchoud@unifr.ch, simon.blanchoud@gmail.com): Due to insufficient funding, I had to close down my lab at the end of April. Although I'm sad not to have the chance to work on Tunicates anymore, I'm mostly grateful to have had the opportunity to study these amazing animals for some years. I would like to take this opportunity to thank everyone in this community, which welcomed me so warmly! I will be looking forward to reading all the great discoveries that you will be making on Tunicates. Go Tunicates! Simon.

8. From Oshrat Ben-Hamo (oshrat.bio@gmail.com): I have been dedicated to the study of *Botryllus schlosseri* for several years now. I specialize in creating scientific illustrations and animations. I would like to share with you a link to my website, where you will find several educational *Botryllus* gif animations available for free download, including blastogenesis gif that eases the understanding of this concept. Further animations are planned and will be added in the future. I would be grateful if you could forward this information to anyone who may be interested. My motive for creating these animations is to raise awareness about this fascinating model animal. I believe that by providing visual and engaging representations of the unique characteristics and behavior of *Botryllus schlosseri*, it is possible to inspire others to develop a deeper appreciation and understanding of this species. The animations are free to download: <https://www.science-art-services.com/free-botryllus-illustrations> or <https://oshratbenhamo.wixsite.com/oshier/free-botryllus-illustrations>.

Work in Progress

1. From Lucia Manni (lucia.manni@unipd.it): New Research Topic on "Tunicates in Evolutionary Developmental Biology" to be published in *Frontiers in Ecology and Evolution*.

The Research Topic intends to highlight recent progress in our understanding of the Evo-Devo of tunicates with attention to key studies that have probed the development of various cell/tissue/organ types in tunicates in the context of chordate evolution. The Topic, Edited by Chiara Anselmi (Stanford University), Rosa Maria Sepe (Stazione Zoologica A. Dohrn, Naples), Lucia Manni (University of Padova), and Paolo Sordino (Stazione Zoologica A. Dohrn, Naples), aims to gather contributions from Evo-Devo researchers in the fields of solitary and colonial tunicates such as, for example: whole body regeneration and other regenerative processes; evolution of stem cells, immune system and other systems; variation in genetic pathways between divergent tunicates despite the morphological similarities; comparison of different developmental pathways in the same organism (e.g., sexual and asexual); environmental effects on developmental trajectories and their evolutionary implications; gene networks regulating phenotypes by integrating cellular, molecular, developmental, behavioral and morphogenetic processes with Omics approaches.

Abstract submission deadline was set at 01 May 2023 and manuscript submission deadline 30 September 2023, but please write to Lucia Manni if you wish to participate.

Information at <https://www.frontiersin.org/research-topics/52166/tunicates-in-evolutionary-developmental-biology>

2. From Delphine Dauga (contact@bioself-communication.com): 2023 ANISEED user survey: ANISEED turns 20 this year and it is time for a little revamp to adapt to new experimental and computational technologies. We need your input to make sure what we do fulfills the main desires of the community. You will thus soon receive the links to one or several user surveys. We are fully aware that everyone is super busy, and we would be very grateful if you took some time to answer the surveys. This will not take more than 20-30 minutes and you may even be able to win something!

- scRNA-seq data survey: <https://anis-dev.aniseed.cnrs.fr/survey.html>

We are already working on a pipeline for the aggregation and sharing of heterogeneous ascidian scRNA-seq datasets (as we understood from the discussion during the last Ascidian meeting that the community is quite keen for such an evolution). Our goal is to provide free scRNA-seq data exploration tools, including APIs and interfaces, to query public and perhaps personal datasets from once place and explore them visually. To fulfil this goal we would like to ask you:

- about the data file format you use & your pipeline (if you intend to generate scRNA-seq data and include them in ANISEED)

- about the different features you would like to see included in the mining tools we will develop (if you intend mining ANISEED datasets)

We appreciate you taking a couple of minutes to answer these questions here:

<https://tinyurl.com/aniseedscrnaseq> We look forward to your input on this point!

- 2023 call for financial contributions to ANISEED

As you probably know, we would like to encourage individual labs to freely contribute to the funding of the curation/maintenance work, in addition to general maintenance of the system and new tool development. Hosting individual lab data on ANISEED is also a good way to ensure compliance with the FAIR guidelines (https://en.wikipedia.org/wiki/FAIR_data). As such we encourage labs to include some ANISEED FAIR subcontracting in their grant applications. We can provide you with a standard paragraph you could use/adapt to your needs.

Procedure for contributing to ANISEED's funding:

- Depending on the finance rules of your institution, you may or not be able to make a straight donation. If you can, this is the easiest. If you cannot, we can send you an invoice. For this, we need to identify an item we could bill you (e.g. the curation and display of one of your articles, hosting or annotating a genome for your favorite species ahead of after publication, permanent link to supplementary or main text data in a paper, etc.).
- If necessary, I can also prepare a quote, and to justify the payment, I can send you a report of the work done with your contribution. To simplify the procedure from your end, you're welcome to put me in contact with your finance officer.

Thanks to your annual contribution, we have been able to continue to work with Marion Gueroult-Bellone (who is currently halfway through her contract) who entered since last November, about 20 articles, with more than 1000 experiments and more than 200 regulatory regions analysis. We also occasionally work with Lea Girard, a highly motivated self-employed (micro)biologist turned ascidian developmentalist for the occasion. Thank you in advance for your contribution, which is crucial for the continuation of our curation work.

If you want your lab to be listed in the Tunicate Portal (<http://tunicate-portal.org/life-community/ascidian-labs>), please fill in this small form (no more than 2 minutes to fill it in): <https://forms.gle/N3nfJbyTQzB3qnWA8> As a reminder : The tunicate portal is a directory of all resources (databases and standards) on tunicates. Please contact me if something is missing! Why a directory of our labs? because we are a small and friendly community, and having a directory of all the labs makes it easier to collaborate!

3. From Loriano Ballarin (loriano.ballarin@unipd.it): Dear all, I am coordinating a special issue of "Marine Drugs" entitled "Bioactive Natural Products from Ascidiaceans and Its Symbionts". I have eight full waiver slots. I invite all of you interested in publishing in this issue to submit your manuscript. Best regards, Loriano.

4. From Howard Jacobs (howard.jacobs@tuni.fi)

I am a mitochondrial biologist based in Finland, working mostly with flies, mice and mammalian cells. However, we have made some use of *Ciona* in recent years, as a source of the alternative oxidase (AOX) gene, for our studies on mitochondrial function and dysfunction. We have now turned our attention more to the mitochondrial role in heat production. One question is whether AOX is used physiologically to regulate the balance between ATP and heat production, especially in cold environments. For this study the antarctic species *C. antarctica* might be suitable, but there is little published data that I could source, other than on its morphology, and no deposited DNA or protein sequences. Are any of you working on this species or aware of someone else who is? And, if so, where does it fit into the phylogeny of the genus - is it believed to be closer to *C. robusta* or *C. intestinalis*, or distant from both? Looking forward to hearing from someone, Howy Jacobs (Tampere, Finland).

Meetings Abstracts

1. European SETAC meeting, Dublin, May 2023.

Bad Romance- The Relationship between Microplastic and Filter-Feeders. Eden Harel, Ines Zucker, and Noa Shenkar (shenkarn@tauex.tau.ac.il) .

Plastic pollution is a growing threat to marine organisms and ecosystem. Once the plastic litter reaches the ocean it is prone to degradation due to forces such as UV, water turbulence, heat, and biological activity. This increases the particles' surface roughness and porosity which causes the

decrease in particle size to micro sized particles (MP). As the size of the particles decreases the range of organisms able to ingest the particles increases. MP are often transported from the water column to marine food webs by filter-feeder organisms which play an important role in benthic-pelagic coupling. The ingested MP may accumulate in the gut or may pass through the digestive system where they are subject to different pH fluctuations and to various digestive enzymes, ultimately excreted within the organism's feces. Feces sink down the water column transporting carbon to deeper waters, therefore acting as a vector of MP through marine zones. Ascidiaceae (Chordata, Ascidiaceae) are efficient filter-feeders, able to remove minute particulate matter from the water column. Therefore, they present fundamental opportunities for understanding the interaction of plastic debris with marine organisms. Here, we use engineered MP particles of high environmental relevancy, to examine the consequences of transit through an organism's digestive system. We examined the changes of the particles' surface area, size, morphology and surface functionality. Particles were fed and later extracted from the feces of two solitary ascidian species: *Styela plicata* and *Polycarpa mytiligera*. Additionally, we measured the effect of the MP on gamete quality, fertilization and early development of the ascidian *Herdmania momus*. Our preliminary results reveal that the extracted MP (following digestion by ascidians) had a rough surface area coated by organic matter. This change in surface area and functionality may later influence the MP availability to other organisms. Currently, we examine how ascidians clear MP sized between 60 μm and 0.45 μm from the water column. Moreover we expose gametes to MP treatments examining the fertilization success. Reproduction by spawning and external fertilization is very common among invertebrates; in the open water gametes are vulnerable to MP and other pollutants. Overall, as MP transfer through marine food webs across marine ecosystems, it is essential to promote the understanding of the consequences of biological digestion.

2. XXIII Meeting of the Italian Association of Developmental and Comparative Immunobiology, Naples, Italy, February 13-15, 2023.

a) Franchi N., Ballarin L., Cima F. *Botryllin*, a novel antimicrobial peptide from the colonial ascidian *Botryllus schlosseri*.

By mining the transcriptome of the colonial ascidian *Botryllus schlosseri*, we identified a transcript for a novel styelin-like antimicrobial peptide, we named botryllin. The gene is constitutively transcribed by circulating cytotoxic morula cells (MCs). The synthetic peptide, obtained from in silico translation of the transcript, shows robust killing activity to bacterial and unicellular yeast cells causing breakages of both the plasma membrane and the cell wall. Specific monoclonal antibodies were raised against the epitopes of the putative amino acid sequence: they label the MC granular content. Upon MC degranulation induced by the presence of nonself, the antibodies recognise the extracellular nets with entrapped bacteria nearby MC remains. The obtained results suggest that the botryllin gene carries the information for the synthesis of an AMP involved in the protection of *B. schlosseri* from invading foreign cells.

b) Drago L., Pennati A., Rothbacher U., Santovito G., Ballarin L. *Stress granules-related genes regulation during embryogenesis of an invertebrate chordate.*

Stress granules (SGs) allow eucaryotic cells to regulate stress responses and face adverse environmental conditions. Their formation occurs in the cytoplasm with the over-expression of mRNA binding proteins, the aggregation of which blocks the translation of mRNAs for anti-stress proteins and prolong their stability. The protection from stress represents a critical issue during embryogenesis and is important for survival of the organisms and the perpetuation of the species. The aim of this research was to investigate the transcriptional regulation of the genes for three important protein components of SGs, during early stages of development of the solitary ascidian *Ciona robusta*, the role of which in stress defense was already demonstrated in adult: TIA1 cytotoxic granule associated

RNA binding protein like (TIAR), tristetraprolin (TTP) and GAP SH3 domain-binding protein (G3BP). Electroporation experiments on embryos were carried out with constructs for reporter gene (LacZ) expression, containing the promoter region for TIAR, TTP or G3BP. The gene reporter assays allowed us to study level, time and cellular specificity of the production of TIAR, TTP and G3BP, which reflects the action of the regulatory sequences, especially occurring in notochord and mesenchyme cells of larval stages under normal physiologic conditions and in response to metal-induced stress.

c) Ballarin L. **Ascidian cytotoxic cells: from zero to hero.**

Ascidian cytotoxic cells are multivacuolated cells, variable in morphology, abundantly represented in the circulation, playing important roles in ascidian immunosurveillance. The interest of researchers towards these cells arose three decades ago when it was realized that, in colonial species, they were the effectors of the rejection reaction between contacting, genetically incompatible colonies. Indeed, they are the first cells to sense nonself and, upon the recognition of foreign molecules, are selectively recruited to the infection site where they release the content of their vacuoles. Their cytotoxic activity is closely linked to the activity of the enzyme phenoloxidase (PO), a copper-containing enzyme widely distributed in

invertebrates, contained inside their vacuoles together with its polyphenol substrata. Recent data indicate that ascidian cytotoxic cells synthesize and release the majority of the complement factors of both the alternative and lectin pathways. In addition, they are also the main source of antimicrobial peptides codified by the ascidian genome. Therefore, these cells, once neglected, are now drawing the attention of researchers for their multiple roles in immune defense.

3. 95th National Congress of the Italian Society for Experimental Biology, Trieste, April 12-15, 2023.

Drago L., Pennati A., Rothbacher U., Ballarin L. **Embryogenesis in an invertebrate chordate: a stress granules-related gene tale.** J. Biol. Res, 96 (Suppl. 1): 16.

Stress granules (SGs) are dynamic non-membranous foci formed in the cytoplasm of eukaryotic cells exposed to a stress condition, especially through the aggregation of mRNA-binding proteins. Inside SGs, mRNAs for anti-stress proteins are temporally silenced, allowing the regulation of various cellular processes, especially those involved in cell defence. The protection from stress is a critical issue during embryogenesis, important for survival of the organisms and the perpetuation of the species. The aim of this research is to investigate the transcriptional regulation of the genes for TIA1 related nucleolysin (TIAR), triste-traproline (TTP) and GTPase activating binding protein (G3BP), important molecular markers of SGs, during early stages of development of the solitary ascidian *Ciona robusta*. Electroporation experiments on embryos were carried out with constructs for reporter gene (LacZ) expression, containing the promoter region of *tiar*, *ttp* or *g3bp*. The gene reporter assays allowed us to study level, time and cellular specificity of the transcription of the studied genes, which reflects the action of the regulatory sequences, especially occurring in mesenchyme of larval stages, under both normal physiologic conditions and in response to metal-induced stress.

4. Benthic Ecology Meeting, Miami, Florida April 26-29, 2023.

a) **Happy at home: distinct microbial communities among microhabitats in an ascidian-crustacean complex.** Brenna Hutchings¹, Susanna López-Legentil¹, Lauren Stefaniak², Marie Nydam³, Patrick M. Erwin¹. ¹Dept. of Biol. & Mar. Biol., Center for Mar. Sci., Univ. of N. Carolina Wilmington; ²Dept. of Mar. Sci., Coastal Carolina Univ., Conway, S. Carolina; ³SOKA Univ. of America, Aliso Viejo, CA.

Ascidians (Chordata: Tunicata) are marine invertebrates known to occasionally host symbiotic crustaceans. While both the microbiomes of ascidians and amphipod crustaceans of various species have been characterized, there is limited documentation of microbial communities in an ascidian-crustacean complex. In this study, four replicates of the widespread ascidian species *Ascidia sydneiensis* hosting symbiotic crustaceans in their branchial sacs were collected from Thunderbirds Marina in Belize. Replicates were analyzed to determine similarities between the microbiomes associated with the ascidian host (tunic and branchial sac regions), symbiotic crustacean, and surrounding seawater. We sequenced a fragment (v4 region) of the 16s rRNA gene and analyzed the resulting 588,037 sequences in the mothur software package and MicrobiomeAnalyst. Microbiome richness (alpha-diversity, $p=0.0048$) and similarity (beta-diversity, $p=0.001$) differed significantly between each sample type, with crustacean microbiomes exhibiting low richness and greater compositional similarity to host microbiomes in the branchial sac than tunic regions or ambient seawater. Similarly, OTU-level analyses revealed that only 3 out of 1,581 OTUs differed significantly ($p<0.05$; $FDR<0.05$) in relative abundance between the crustacean and branchial sac microbiomes, compared to 25 OTUs between the crustacean and tunic region, and 208 OTUs between the crustacean and ambient seawater. Together, these data show that different body regions of *Ascidia sydneiensis* represent distinct microhabitats with specific microbiomes, and that symbiotic crustaceans therein contain microbiomes matching the host microhabitat they occupy. High similarity between the microbiomes of the amphipod crustacean and the branchial sac of *Ascidia sydneiensis* may indicate environmental filtering of microbial communities sharing a common microhabitat or microbial trafficking from host to symbiont. Future studies examining generalist and host-specific crustaceans are needed to establish determinants of microbiome composition within microhabitats of ascidian-crustacean complexes.

b) Evolving together: Microbial symbionts as indicators of speciation. Susanna López-Legentil, James S. Evans, Samantha K. Morrison, Patrick M. Erwin.

Ascidians exhibit morphological plasticity and host-specific microbial symbiont communities. The colonial ascidians *Distaplia bermudensis* and *Polyclinum constellatum* occur in a wide range of color morphs yet showed no diagnostic differences in zooid morphologies. To determine whether color variation had a genetic basis, we sequenced fragments of the 18S rRNA and COI genes. We also obtained partial 16S rRNA gene sequences for microbiome characterization of common color morphs. In *D. bermudensis*, phylogenetic analyses revealed two genetic lineages with distinct microbiomes that corresponded to unique color morphology groupings, indicating that two distinct species coexist under the same scientific name. In contrast, for *P. constellatum*, a single genetic lineage included all color variants with no significant differences among their microbial communities, indicating that color variation is solely due to phenotypic plasticity. Our study demonstrates the utility of symbiont profiling in resolving the taxonomic status of phenotypically variable species and highlights the host species-specific nature of cryptic speciation events.

c) All in the mix: Functional profiles of ascidian microbiomes remain stable despite shifts in symbiont composition. Patrick M. Erwin, Brenna Hutchings, Susanna López-Legentil.

Ascidians are home to diverse microbial symbiont communities ('microbiomes') that contribute to host physiology and fitness. Compositional shifts in ascidian microbiomes may therefore impact host success in different habitats and mediate the environmental tolerance of the host. Fundamental to this hypothesis are implicit structure-function relationships in the microbiome, namely that changes in taxonomic composition will alter community functionality. In this study, we conducted functional microbiome profiling of a native ascidian host (*Eudistoma capsulatum*) previously shown to exhibit taxonomic shifts in microbial symbionts across natural (reef) and artificial (harbor) habitats in North Carolina. Taxonomic and functional annotation of shotgun metagenomes confirmed compositional shifts in the microbiome of *E. capsulatum* across habitats and revealed gene content associated with diverse metabolic pathways in carbon, nitrogen, and sulfur cycles. A high prevalence of genes related

to amino acid biosynthesis and degradation were detected, coding for enzymes involved in the metabolism of 9 essential and 9 non-essential amino acids. Other notable gene features were associated with vitamin biosynthesis and the biodegradation of xenobiotics, including herbicides and persistent organic pollutants. This functional microbiome signature was consistent in ascidians from both reef and harbor locations, even at fine-scale levels: only 1% of the >2,400 individual genes examined differed significantly between habitats. Together, these findings show that habitat-driven compositional changes in the microbiome of *E. capsulatum* do not manifest in altered functionality. Rather, functional redundancy among microbiome members appears to maintain gene content despite membership turnover. Such functional convergence on important microbiome features identified herein (e.g., host metabolism and pollution tolerance) may represent an adaptive mechanism for ascidian survival and colonization of new locations. Additional studies that target functional profiling of introduced ascidians and incorporate gene expression analyses are needed to determine the relationship between symbiont function and host distribution.

Thesis Abstracts

1. Migration and microbiome in *Botrylloides diegensis*. Margaux de Raemy, UNIFR (Université de Fribourg, Switzerland). Advisor Dr. Simon Blanchoud. simon.blanchoud@unifr.ch

Botrylloides diegensis are marine invertebrates that our group studies mainly for their incredible capacity for regeneration. *B. diegensis* have a biphasic life cycle, as a free-living larva and as a sessile colony attached to some substrate. Although *B. diegensis* are described as sessile, we have nevertheless suspected movements of colonies in our laboratory, which is why I decided to study their migration in more details. During my experiments, I also observed that colonies cultured in freshly prepared artificial seawater (ASW) quickly underwent a stress reaction known as regression. I suspected that the absence of a suitable microbiome in the seawater, which they filter for feeding, was influencing their development. I decided to study the environmental microbiome of *Botrylloides diegensis* as a second topic. This thesis starts with an introduction on *Botrylloides diegensis*, their morphology, life cycle as well as an introduction on migration and microbiomes. Then the materials and methods section describes all the tools used in our experiments. In the results and hypothesis, I present my data on how and why *B. diegensis* migrate as well as my results on our microbiome experiments. In the discussion, I conclude this thesis with the interpretation of my results, an analysis of the difficulties experienced during experiments and with a few future perspectives on how to continue these projects. Overall, in my master thesis I have shown that *B. diegensis* migrate consistently upwards, against the direction of gravity, at an average speed of 1.15 mm/day and that the environmental microbiome can have either a positive or a negative influence on the survival of the animals, depending on the concentration and the species of the inoculated bacteria.

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