ASCIDIAN NEWS*

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Number 80 December 2017

It was a very busy summer for me, ascidiologically speaking! Rosana Rocha and I taught a 2 week tunicate taxonomy and biology workshop June 20-July 4 in Panama, at the Smithsonian's Bocas del Toro Tropical Research Institute on the Caribbean. This was the 5th advanced workshop we have taught since 2006 at this lab; it is very gratifying to see that many of the participants are now faculty members at various institutions worldwide, with their own labs and students pursuing research projects on ascidians, some of whom are now participants in these workshops.

A few days at home, then off to New York City for the 9th Intl. Tunicata meeting in mid-July at New York University, hosted by Dr. Lionel Christiaen. It was fun to see so many colleagues and exciting to hear about the latest research. See more about the meeting below, and in the Meeting Abstracts section where you will find a link to the downloadable program and abstracts.

Only a day and a half back home, then to British Columbia to participate in a 3 week marine biodiversity survey at the Hakai Institute on remote Calvert Island with a number of other taxonomists. Many surprises! Read about it in the Work in Progress section below.

A big thank-you to all who sent in contributions. There are **101** New Publications listed at the end of this issue. Please continue to send me articles, and your new papers, to be included in the next issue of AN.

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

NEWS AND VIEWS

1. The **next Intl. Invasive Sea Squirt Conference (IISSC)** will be **May 2-4, 2018** at Woods Hole Oceanographic Institution in Woods Hole, Massachusetts. The registration page for the Conference is now open: https://web.whoi.edu/sea-squirt-conference/. Please submit your abstracts to **Mary Carman** at mcarman@whoi.edu. I look forward to seeing you at the conference! Conference proceedings will be peer reviewed and published in a special issue of the REABIC journal Management of Biological Invasions.

2. From **Evangelina Schwindt** (schwindtcnp@gmail.com):
The **10th International Conference on Marine Bioinvasions** will be held in Puerto Madryn, Patagonia, Argentina, **October 16-18, 2018**. For more information, go to www.marinebioinvasions.info or follow on Facebook @marinebioinvasions, Twitter @ICMB2018 or Instagram. Start planning your trip to Patagonia now! We will be opening the call for Abstracts by March-April 2018.

3. From your AN editor: The **9th Intl. Tunicata meeting, New York City July 17-21** was very well attended with special sessions on Embryogenesis, Developmental Cell Biology, Genomics, Allorecognition and Immunity, Nervous System Development, Evolution and Anatomy, Gene Regulation, Imaging Ascidian Development, Systematics and Evolution, Ecology, Late Embryos and Larvae including metamorphic events, Asexual Reproduction and Regeneration, and Anatomical Ontology.

I had been invited to give a keynote talk in the Systematics session; when I finished, the organizers had a big surprise; they presented me with this beautiful large crystal plaque for my lifetime achievements in ascidian biology and for producing Ascidian News, which has helped to keep our worldwide ascidian community together for 42 years! I was totally overwhelmed. This is the first time such an award has been given at these meetings. My sincere thanks to all for this huge honor.

Anna Di Gregario, Gretchen, and Billie Swalla

4. From **Billie Swalla (**bjswalla@uw.edu**)** (with help from Anna, and Lionel Christiaen): **Gretchen Lambert wins the Tunicate Award at the Ninth International Tunicate Meetings**

Gretchen Lambert won a richly deserved “Tunicate Award” at the Ninth Intl. Tunicate meetings that were held in New York City at New York University in July, 2017. Gretchen is a world renowned Tunicate Taxonomist, who has been working on ascidians for over 50 years now. Of course, we know Gretchen as the person who compiles Ascidian News, which she began with her beloved husband, Charley Lambert in 1975. Gretchen has had a long and illustrious career working on ascidians, publishing 73 papers on many topics: biogeography, ecology, invasions, taxonomy, biomineralization and even some about fertilization, with Charley. In 2005 she identified and catalogued the entire ascidian collection of the Santa Barbara Museum of Natural History and has also donated her personal large collection to that museum, another wonderful service she has done for the Tunicate Community. Earlier this year, Gretchen again co-taught the “Biology and Taxonomy of Ascidians” workshop at the Smithsonian Bocas del Toro lab in Panama with Rosana Rocha, educating an entire new group of students and postdocs to tunicate biology. Gretchen has served as a consultant to numerous biologists, aquaculturists and agencies around the world. She continues to work on assessing ascidian biodiversity and invasion issues, and thus additionally their importance in community ecology, conservation and restoration. For all of these accomplishments, and for being an enthusiastic and informed member of the International Tunicate Community for all these years, we thank and congratulate Gretchen Lambert for her outstanding tunicate science.

5. From **Stefano Tiozzo** tiozzo@obs-vlfr.fr

In order to help us to organize the 10th Intl. Tunicata Meeting we aim to decide which week would work better. We would also propose a few changes to the classic ITM format:

a) The possibility to abolish thematic sessions and adopt a “no session format”, where different talks can mix, exposing everybody to the different aspects of tunicate biology.

b) Introduce a half-day sessions focused on developmental biology, ecology, regeneration and immunology in non tunicate models. Speakers from outside the community would be invited.

c) There may be the possibility to organize a two day workshop on imaging and transgenesis in tunicates, before or after the ITM.

So we are asking to respond to a short survey by clicking the link below at your earliest convenience.
https://fr.surveymonkey.com/r/3KDWSM8

6. From **Delphine Dauga** (contact@bioself-communication.com)

The new ANISEED update paper published in the upcoming 2018 database issue of Nucleic Acids Research is now online:

- Abstract:
https://academic.oup.com/nar/advance-article-abstract/doi/10.1093/nar/gkx1108/4628129
- Article (free access):
https://academic.oup.com/nar/advance-article/doi/10.1093/nar/gkx1108/4628129?guestAccessKey=708adf92-7e7b-4322-a443-3332c522177d

Thanks to the work of the Aniseed team and of many contributors from the community, the new system has been significantly improved. Some highlights:

- A new section describing the species covered, and linking to the molecular, but also environmental and taxonomy databases in the field.
- A brand new genome browser, the WashU epigenome browser. This browser offers many new functionalities and avoids all the bugs some of you may have experienced in the past with Gbrowse. It is also perfectly adapted to the upcoming HiC chromatin conformation data.
- A novel synteny browser, Genomicus, a rich resource to explore the conservation of gene order within tunicates and with vertebrates.
- Three new functional genome annotations: Molgula oculata, Halocynthia aurantium and Phallusia fumigata.
- A novel orthology pipeline (which will keep improving over the next couple of months), and interactive phylogenetic trees for a majority of tunicate genes.
- A novel RNA-seq section in the expression tab of each Ciona robusta, Phallusia mammillata and Halocynthia roretzi gene.
- A novel SELEX-seq tab for 140 ciona robusta transcription factors and around 80 Phallusia orthologs. You will also find a public hub on the WashU epigenome browser showing you predicted local TF binding affinity across the *Ciona robusta* and *Phallusia mammillata* genomes.
- An extended download section
- Many new articles curated by Delphine and her team, with the help of community members.

Aniseed will continue to improve over the next couple of years, with some strategic changes. In particular, as the stream of [your!] data is increasing and in order to offer traceability of query results, we will implement a “release" philosophy: Each release of the database will be stable for 6 months, before being archived and replaced by a new release. Also, the "3D virtual embryo" will soon be back, only much, much more powerful and simple to use than the first one!

We hope you will like the new Aniseed, and remember: a small donation is most welcome to keep us going. You can now follow ANISEED on Twitter: @ANISEED_DB and Facebook: https://www.facebook.com/TunicateCommunity

WORK IN PROGRESS

1. From **Gretchen Lambert**: In late July I participated in a 3 week intensive marine biodiversity survey along with many other taxonomists at the Hakai Institute on very remote Calvert Island in northern British Columbia. From Vancouver we took a charter flight to Bella Bella, BC followed by a 1½ hour boat ride around and between numerous islands to our destination. I expected a tiny rustic lab but it was a truly magnificent, beautiful facility. It had formerly been a fishing lodge with very comfortable accommodations that was purchased several years ago by the Tula Foundation and renamed the Hakai Institute, which "conducts long-term scientific research at remote locations on the coastal margin of British Columbia, Canada." (https://www.hakai.org/) This survey (officially known as the 2017 Hakai MarineGEOBC BioBlitz) was one of the more intensive surveys being carried out this year to celebrate Canada's 150th anniversary of its independence, by recording the country's amazing terrestrial, aquatic and marine biodiversity especially in remote areas. Many new species are being described as a result, and our Hakai survey was no exception. The survey included daily sampling by the staff and visiting taxonomists of many marine habitats: rocky and sand/gravel intertidal, eelgrass meadows, shallow and deeper subtidal by snorkel and scuba, and artificial surfaces of settlement plates set out up to a year ago, and the bottom of the large floating dock at the Institute. In just this small area I identified 37 ascidian species, including 3 new species, which represents about 1/3 of all the known North America species from Alaska to southern California. Taxonomists working on other groups reported similar amazing diversity in this tiny Hot Spot. Remarkably, of the 37 spp. of ascidians, only 1 is a possible non-native, *Diplosoma listerianum*, and it was collected only on natural substrates including deeper areas sampled by scuba. There were no botryllids, no *Styela clava*, no *Didemnum vexillum*, though these are all common in other parts of BC. I had wondered what species I would encounter; would they be similar to the Alaska fauna, or to the Washington or California fauna? Would non-native species have invaded even this remote area, with no commercial shipping and only a small amount of recreational-size boat traffic? Most of the species are the same as in northern California, Washington, and southern BC, with only a small overlap of a few of the known Alaska spp.

It was a challenging, exciting, exhausting 3 weeks! Long hours at the microscope. I will be writing up a paper on the ascidians, but with 3 new species to describe it will take a while.

2. From **Patrick Lemaire**, CRBM, 1919 Route de Mende, F-34293 MONTPELLIER Cédex 5, France. (patrick.lemaire@crbm.cnrs.fr)

Following the final round table of the ITM9 meeting in NYC, you may have noticed that we have now replaced the species name *Ciona intestinalis* with *Ciona robusta* in most ANISEED datasets. ***This means that you now need to search Ciona robusta datasets to obtain the results you used to obtain with a Ciona intestinalis search.*** For the datasets generated in Japan, the US and probably Italy, where *Ciona robusta* has been (unknowingly) used for decades, this is an adequate change, which agrees with the new species assignment.

Datasets generated in Eastern Canada and Northern Europe, however, are mostly using the "real" *Ciona intestinalis* (formerly type B), for which we only have a very poor genome assembly, too poor to generate gene models. Until a decent *Ciona intestinalis* genome assembly is produced, ANISEED will map all datasets coming from either Cionas onto the *C. robusta* genome, and the *Ciona intestinalis* section will be limited to a description of the embryonic anatomy and to a link to the - currently poor and unannotated - genome assembly.

Besides the issue of mapping all datasets onto the right species, an improved genome for *Ciona intestinalis* would open numerous new comparative genomics research avenues. As an example, Northern European *Ciona intestinalis* shows a much higher haplotype diversity than *Ciona robusta*, which suggests that comparative population genetics of the two species will be fascinating. During the ITM9, Nori Satoh and I discussed the issue of *C. intestinalis* genome sequencing/assembly. Nori offered to generate sufficient raw sequences (both short and long reads) to construct a good assembly, provided someone volunteers to assemble these data. We, at ANISEED, do not currently have sufficient resources to assemble this genome, but we would be happy to build and functionally annotate a gene model set for *Ciona intestinalis*, and create a dedicated browser. This leaves open the assembly of the sequences into scaffolds. It would be wonderful if someone in the community would volunteer to take on this step. A realistic aim would be to generate an assembly with an N50 scaffold size ~100kb, and a an N90 size in the ~10kb range, <5% Ns and few misassemblies.

It you are interested in the challenge, please contact either Nori or myself.

Best wishes, Patrick http://www.crbm.cnrs.fr/index.php/en/patrick-lemaire-uk

3. On behalf of the journal Developmental Biology, the guest editors are pleased to announce a call for papers for a special issue "Current Directions in Tunicate Biology", initiated at the 9th International Tunicate Meeting in New York. Please follow the link below for details: https://www.journals.elsevier.com/developmental-biology/call-for-papers/special-issue-on-current-directions-in-tunicate-biology

Please direct any questions to: **Steve Irvine** sirvine@uri.edu. The other guest editors are Anna Di Gregorio adg13@nyu.edu, and Filomena Ristoratore filomena.ristoratore@szn.it

4. From **Andrea Moore** (Andrea.Moore@dfo-mpo.gc.ca)

Fisheries and Oceans Canada has developed a screening-level risk assessment tool for marine non-indigenous species called CMIST. CMIST has been tested, peer-reviewed, and recommended for use with marine invertebrates, including tunicates. It contains 17 questions on likelihood of introduction and impact of invasion for a species in a specific assessment area and generates risk scores that incorporate uncertainty. A database with existing assessments is now available online and the tool is available for download and use. http://www.bio.gc.ca/science/monitoring-monitorage/cmist/index-en.php.

DFO (2015) Marine screening-level risk assessment protocol for marine non-indigenous species. DFO Canadian Science Advisory Secretariat Science Advisory Report 2015/044

Drolet D, DiBacco C, Locke A, McKenzie CH, McKindsey CW, Moore AM, Webb JL, Therriault TW (2016) Evaluation of a new screening-level risk assessment tool applied to non-indigenous marine invertebrates in Canadian coastal waters. *Biological Invasions* 18: 279–294

ABSTRACTS FROM RECENT MEETINGS

1. 9th Intl. Tunicata Meeting, New York University, New York, NY July 16-21, 2017.
Links to the entire downloadable program and all the abstracts can be found at https://2017-tunicate-meeting.bio.nyu.edu/program/.

2. PanAm EvoDevo meeting, University of Calgary, Calgary, Alberta, Canada, August 19-23, 2017.

a) Molgulid tales. Billie J. Swalla, Univ. of Washington Friday Harbor Laboratories, Friday Harbor, WA bjswalla@u.washington.edu

Transcriptome and genome data offer an exciting new approach to examine the origin and evolution of the chordate body plan. We've studied chordate body plan evolution with two tunicate species with radically different larval body plans that are found sympatrically off the coast of Roscoff, France -*Molgula oculata* and the tailless *M. occulta.* Tailed *M. oculata* larvae have tail muscle cells flanking the notochord in the tail, and in the head is the otolith, a gravity sensory organ. The tailless *M. occulta* does not form a tail in their larval stage, and have only twenty notochord cells that do not converge and extend during larval development. We have sequenced the genomes of these two species and a third species, *M. occidentalis* in collaboration with the Christiaen lab, and they are available on Aniseed (Stolfi et al. 2014). We show by transcriptome and in situ hybridization analysis that the notochord gene network is expressed at the right time and place in the tailless *M. occulta* embryos and larvae, although the notochord collapses into a "notoball" near the posterior. We show by transcriptome analyses that the ascidian metamorphosis program begins much earlier in molgulid ascidians, during early development. This radical heterochronic shift has been documented in another tailless ascidian, *Molgula tectiformis*, and is now reported for three additional species: the tailed molgulid species, *Molgula oculata, Molgula occidentalis,* and the tailless *Molgula occulta*. Further functional data is necessary to determine if this pronounced heterochrony is the necessary preadaptation for tailless tadpole to evolve in molgulid ascidians. This is an excellent model system to study the evolution of gene networks underlying morphology.

Stolfi, A., Lowe, E., Racioppi, C., Ristoratore, F., Swalla, B.J., Brown, C.T. and Christiaen, L. (2014) Divergent mechanisms regulate conserved cardiopharyngeal development and gene expression in distantly related ascidians. *eLife* 2014:3:e03728

b) Coloniality in marine chordates: eco-evo-devo approaches to understand different levels of biological organization. Federico Brown, Departamento de Zoologia, Universidade de São Paulo, Brazil. fdbrown@usp.br

We have undertaken a comparative and multidisciplinary approach to link cellular and molecular understanding of development to the evolution of a major evolutionary transition, i.e. the evolution of coloniality. The Tunicata, sister group of vertebrates, contains clades with solitary species that reproduce only sexually, or colonial species that reproduce both asexually and sexually. These clades have evolved multiple times independently. Whereas solitary forms have evolved tougher tunics and individuals are generally larger to withstand predators, colonial forms can grow rapidly and directional growth may allow them to grow into spatial refuges free of predators. First, we conducted a three-month-long manipulative field experiment under different ecological treatments to determine how biotic factors influence the development and growth of solitary or colonial species. In these studies, competition with other organisms did not show consistent effects between life forms, however predation showed a significantly higher effect on the growth of colonial species. Second, we hypothesized that the ability of colonial tunicates to reproduce clonally by budding may be related to the remarkable potential of regeneration and a high evolvability in the mechanisms that regulate adult stem cell development. We compared budding mechanisms in three colonial (i.e. Botryllus schlosseri, Symplegma brakenhielmi, Polyandrocarpa zorritensis) and one solitary species (i.e. Styela plicata) of the same tunicate clade (i.e. Styelidae). All colonial species differed in budding modes, blood cell types, and in the degree of integration and/or synchronization of developmental modules, whereas S. plicata presented a high regenerative potential mediated by circulatory progenitor cells. Similarities and differences in progenitor cells and tissues involved in regeneration or developmental processes of budding among the three colonial species have allowed us to identify potential developmental mechanisms responsible for the evolution of coloniality in this group. Third, we sequenced and continue to assemble the genomes of several colonial species of tunicates to understand genomic changes during the transitions to asexual reproduction, clonality, and coloniality. We are specifically interested in how ncRNAs may have played a role in the regulation of progenitor cells involved in

budding and asexual reproduction. We have already found a pool of candidate miRNAs and other ncRNAs that are overrepresented in the genomes of colonial species that need additional validation and further experimentation in developmental studies. We have just begun to unravel some of the evolutionary forces and developmental changes that may have been responsible for the evolution of coloniality in our own phylum.

3. 29th Cell and Developmental Biology Meeting, Kobe, Japan, October 19-20, 2017.

Mavericks, new models in developmental biology. Billie J. Swalla, Friday Harbor Laboratories and Biology Dept., University of Washington, Seattle, WA, USA. bjswalla@u.washington.edu

The Swalla Lab studies two different chordate model systems to understand recent and distant evolutionary changes in body plan. We study closely related molgulid ascidian species with very different larval phenotypes to understand how gene network function is lost evolutionarily. We show in this system that tail muscle and sensory vesicles are lost in the tailless species, although pseudogenes for melanogenesis are still being expressed and expect that with time these will be down regulated. In contrast, the notochord gene network is intact, even though the tailless species never makes a notochord. We have also been studying ptychoderid hemichordates in order to understand evolution of the chordates from a deuterostome ancestor. Hemichordates have all of the chordate characters except a notochord. The hemichordate *Ptychodera flava* also has amazing capacity to regenerate in both larval and adult life stages. We have used this system to better understand regeneration and are looking for stem cell populations in the hemichordate larvae and adults.

4. 1st Intl. Conference of TWAS Young Affiliates Network, Aug 22-24, 2017, Rio de Janeiro, Brazil.
Evolutionary origins of stem cells. Federico D. Brown, Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil fdbrown@usp.br

Only some animals have the capacity to propagate clonally by budding and form colonies. Within the chordates, tunicates (the sister group of vertebrates) are a marine group of invertebrate animals that have evolved multiple times independently solitary species that reproduce only sexually, or colonial species that reproduce both asexually and sexually. We have undertaken a comparative and multidisciplinary approach to link cellular and molecular understanding of development to macroevolutionary events. We hypothesize that the ability to reproduce clonally by budding is related to the remarkable potential of regeneration in tunicates and a high evolvability in the mechanisms that regulate adult stem cell development. We compared budding mechanisms in three colonial (i.e. Botryllus schlosseri, Symplegma brakenhielmi, Polyandrocarpa zorritensis) and one solitary species (i.e. Styela plicata) of the same tunicate clade (i.e. Styelidae). All colonial species differed in budding modes, blood cell types, and in the degree of integration and/or synchronization of developmental modules, whereas S. plicata presented a high regenerative potential mediated by circulatory progenitor cells. Similarities and differences in progenitor cells and tissues involved in regeneration or developmental processes of budding among the three colonial species have allowed us to identify potential developmental mechanisms responsible for the evolution of coloniality in this group. To understand genomic changes during the transitions to asexual reproduction, clonality, and coloniality, we sequenced and continue to assemble the genomes of several colonial species of tunicates. We are specifically interested in how ncRNAs may have played a role in the regulation of progenitor cells involved in budding and asexual reproduction. We have already found a pool of candidate miRNAs and other ncRNAs that are overrepresented in the genomes of colonial species that need additional validation and further experimentation in developmental studies.

5. Brazilian International Congress of Genetics 2017, Sep 12-15, 2017, Aguas de Lindoia, SP.
a) Evolutionary origins of budding and regeneration in marine chordates: studies of cells, genes, and ncRNAs. Federico D. Brown et al., Dept. of Zoology, Instituto de Biociências, Univ. de Sao Paulo, Brazil. fdbrown@usp.br

To understand how major reproductive modes evolve during life history transitions and to explore ncRNA involvement in these transitions, we focus our research in the study of coloniality in ascidians. We compare the blastogenic development (budding) and the genomes of solitary and colonial styelid tunicates. Solitary, social (i.e. individuals or aggregates), and colonial (i.e. integrated individuals within a common tunic) species occur in the Styelidae. Styela plicata is solitary and does not bud and reproduces only sexually but has good regenerative abilities. Symplegma brakenhielmi is colonial and its individuals develop asynchronously within a vascular network, which connects the zooids of the colony. Botrylloides and Botryllus spp. bud in weekly synchronous cycles of blastogenesis and generally develop by evagination of the lateral epidermis of adults. Comparative development of budding in these species shows that coloniality evolved by an integration of developmental modules in the colony. We studied hemocyte progenitors and analyzed proliferation patterns in these closely related species to identify putative circulatory progenitor cells of budding. Budding in ascidians requires a permanent supply of progenitor cells likely regulated by ncRNA pathways. Therefore by comparing the available genomes of colonial ascidians to the genomes of solitary tunicates we have identified candidate ncRNAs putatively involved in stem cell function and blastogenesis. Patterns of expression and developmental function of these ncRNA candidates remain to be done, but will likely reveal important loci involved in the evolution of asexual modes of reproduction and regeneration. Our results support a stepwise modular integration of budding synchrony and communication systems among individuals during the evolution of coloniality, and raise new questions about ncRNA regulation in stem cell function of colonial marine chordates.

b) Circulatory stem cells of styelid ascidians (Tunicata:Urochordata). Juan Jiménez Merino, Federico D. Brown. Dept. of Zoology, Instituto de Biociências, Universidade de Sao Paulo, Brazil.

Coloniality has originated multiple times within tunicate evolution. Stem cells have a relevant role in budding and whole body regeneration processes of colonial species. In order to understand which cellular mechanisms allowed solitary-colonial transitions to occur, we are comparing putative stem cell populations of the blood to identify homologous lineages in colonial ascidians and closely related solitary species. In this study, we characterize the putative stem cells in the blood of *Styela plicata* and *Styela canopus*. These species belong to a solitary clade in close phylogenetic proximity to a colonial clade of the same family, i.e. Styelidae. We used a comprehensive approach to classify blood cells and identify the undifferentiated lineages of the blood. Based on cell morphology, we identified five broad categories in the blood (abbreviations of blood cell types and mean proportions of corresponding blood cells in S. plicata and S. canopus in parentheses): precursor cells (PRs, 0,09, 0,29), hyaline amoebocytes (HAs, 0,09; 0,06), vacuolated cells (VCs, 0,38; 0,06), granular cells (GCs, 0,4; 0,56), and pigmented cells (PCs, 0,04; 0,03). We found that staining procedures, such as fixatives or slide adhesives, affected shape of several cell types. Additionally, we used imaging flow cytometry to characterize blood cell populations according to their light scattering properties. Two populations of cells were gated, based on forward and side scatter values. Images obtained from both groups were analyzed and classified in three clear morphotypes. These morphotypes probably represent PR, VC and GC populations. Further distinction and isolation of potential stem cells will be achieved through the use of molecular markers specific for hematopoietic lineages. We have selected 12 genes involved with cell stemness and hematopoiesis as marker candidates for further identification of cell lineages. Expression studies of these genes will allow us to further characterize blood cell populations in these species. By an exhaustive characterization of blood cells in Styela, we hope to determine homologous blood cells of colonial styelid species currently under study in our

laboratory. Data generated from this study will provide a proper framework for wider comparisons of blood borne stem cells and hematopoiesis in other tunicates.

c) Blood cells in *Symplegma brakenhielmi* and the evolution of coloniality in styelid ascidians. Stefania Gutierrez, Federico D. Brown. Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil: as.gutierrez57@ib.usp.br, fdbrown@usp.br,

Colonial animals are biological systems composed of discrete units (zooids) that are physiologically interconnected and require coordinated development for the colony to function properly. The origins of modular developmental mechanisms that facilitated the evolution of coloniality remain unclear. In the genera Botryllus and Botrylloides (Styelidae:Tunicata), highly integrated colonies evolved from a solitary ancestor. Colonies are composed of zooids that are interconnected through a common vascular system containing a diversity of circulatory blood cells suggested to be involved in the communication between zooids. Cycles of budding in Botryllus are coordinated and synchronized, i.e. undifferentiated cells (UCs) are known to increase in the circulation during morphogenesis, whereas macrophage like cells (MLCs) increase during programmed cell death (PCD) of adult zooids. To reconstruct ancestral states of coloniality we studied Symplegma brakenhielmi, a sister taxon of the botryllids. S. brakenhielmi zooids are embedded in a common tunic and present a similar vascular system as the brotryllids, however development and turnover of zooids occurs asynchronously and in a more independent manner. We classified S. brakenhielmi blood cells by morphology using cytohistological assays and transmission electron microscopy. We found eleven types of hemocytes previously reported to be involved in a diversity of biological processes, including: (a) macrophage like cells (MLCs) involved in phagocytosis and programmed cell death (PCD); (b) undifferentiated cells (UCs) involved in budding and regeneration; (c) morula cells (MC) involved in immune reactions; (d) nephrocytes or pigment cells involved in storage and excretion. We observed mitotic activity in precursors cells by expression of Phospho-Histone H3, and considerable variation in their characteristics and proportion during regeneration. These results suggest a continuous hematopoiesis, and hemocyte differentiation processes in the blood of the colonies. In vivo observations of zooid regression, together with cytological observations of MLCs, DNA damage of cells by TUNEL, positive expression of Annexin-V, and nuclear morphology by flow cytometry, suggest PCD as a main process of zooid turnover in S. brakenhielmi colonies. Our observations suggest that zooids are recycled after resorption, and lifespan of zooids are controlled by endogenous mechanisms of the colony. Therefore in Botryllus and Symplegma colonies communication between modules evolved by specialized migrating blood cells that carry information across the colony, coordinating the turnover of zooids. Modular communication, PCD, and recycling of tissues mediated by MLCs, represent some of the important developmental processes of colonial evolution in the styelid ascidians.

d) The evolution of coloniality in an ascidian family (family Styelidae, phylum Tunicata): a phylogenomic approach. Laurel Sky Hiebert[1]; Alexandre Alie[2]; Stefano Tiozzo[2], Federico David Brown Almeida[1]. [1]Depto de Zoologia, Instituto Biociências, Universidade de São Paulo, Brazil. [2]Laboratoire de Biologie du Développement de Villefranche-sur-mer, Observatoire Océanographique, Villefranche-sur-mer, France. fdbrown@usp.br

Colonial animals, those that are made up of asexually-derived units, have originated in multiple lineages of metazoans, such as corals, bryozoans, and tunicates. The tunicates (Phylum Tunicata) exhibit a diversity of colonial forms with a range of modes of asexual reproduction that are widespread throughout the taxon. Thus, this suggests that coloniality has evolved multiple times within this phylum. However, the number of times that the colonial life style was lost or gained remains uncertain. Here, we focus on a single family of more than 550 species, the Styelidae (order Stolidobranchia), which possesses both solitary and colonial members. Recent phylogenetic studies

of this family using mitochondrial and nuclear markers have produced trees with low resolution and provide mixed results about the number of times coloniality evolved. Here, we take a RNAseq approach to produce a large gene dataset for resolving the relationships among members of this family. We sequenced and assembled 13 new transcriptomes, and along with the few already publically available, we isolated orthologs, and conducted a phylogenetic analysis based on this large dataset. We present a robust phylogeny and our findings on the evolution of coloniality and budding in this ascidian family.

6. IX Latin American Society for Developmental Biology Meeting 2017, Oct 9-13, Medellín, Colombia.

Evolution and development of coloniality in our own phylum. Federico Brown fdbrown@usp.br. . Dept. of Zoology, Instituto de Biociências, Universidade de Sao Paulo, Brazil.

Tunicates, the sister group of vertebrates, have evolved multiple times independently solitary species that reproduce only sexually, or colonial species that reproduce both asexually and sexually. We have undertaken a comparative and multidisciplinary approach to link cellular and molecular understanding of development to macroevolutionary events. We hypothesize that the ability to reproduce clonally by budding is related to the remarkable potential of regeneration in tunicates and a high evolvability in the mechanisms that regulate adult stem cell development. We compared budding mechanisms in three colonial (i.e. Botryllus schlosseri, Symplegma brakenhielmi, Polyandrocarpa zorritensis) and one solitary species (i.e. Styela plicata) of the same tunicate clade (i.e. Styelidae). All colonial species differed in budding modes, blood cell types, and in the degree of integration and/or synchronization of developmental modules, whereas S. plicata presented a high regenerative potential mediated by circulatory progenitor cells. Similarities and differences in progenitor cells and tissues involved in regeneration or developmental processes of budding among the three colonial species have allowed us to identify potential developmental mechanisms responsible for the evolution of coloniality in this group. To understand genomic changes during the transitions to asexual reproduction, clonality, and coloniality, we sequenced and continue to assemble the genomes of several colonial species of tunicates. We are specifically interested in how ncRNAs may have played a role in the regulation of progenitor cells involved in budding and asexual reproduction. We have already found a pool of candidate miRNAs and other ncRNAs that are overrepresented in the genomes of colonial species that need additional validation and further experimentation in developmental studies. Finally, we carried out a three-month-long manipulative experiment in the field under different ecological treatments to determine how biotic factors influence the development and growth of solitary or colonial species. In these studies, competition with other organisms did not show consistent patterns between life forms, however predation showed a significantly higher effect on the growth of colonial life forms. Altogether, these results have begun to generate several lines of evidence that point to specific evolutionary forces and developmental changes that were responsible for the evolution of coloniality in our own phylum.

THESIS ABSTRACTS

The role of viruses within metaorganisms: *Ciona intestinalis* as a model system. Brittany A. Leigh, Univ. of South Florida, Tampa, FL brittany.a.leigh2@gmail.com. Ph.D. thesis. Graduate Advisors: Larry Dishaw and Mya Breitbart, USF.

The study of the dialogue between animal immunity and associated microbes is of considerable importance and has significant implications for the medical sciences. Animal-associated microbiomes include bacteria, viruses and fungi that vastly outnumber host cells, especially within the gut environment, and are considered to be integral components of most healthy, functioning animals

(i.e., metaorganisms). However, the processes underlying the initial establishment and stabilization (i.e., homeostasis) of these microbial communities are not very well understood. My dissertation focused on the further establishment of a well-known developmental model system, *Ciona intestinalis*, to study host-microbiome dynamics within the gut. A new and effective model for studying microbial colonization of the gut requires the ability to rear germ-free (GF) animals. In my work, I describe the establishment of a GF technique for rearing *Ciona* and some of the basic methods utilized for bacterial exposure and colonization. Additionally, to determine the spatial structure of the gut microbiome, viral and bacterial communities within the three main gut compartments of wild *Ciona* were assessed using viral metagenomics and 16S rRNA gene sequencing, respectively. Most viruses were found to be phages (viruses infecting bacteria), and numerous sequences of prophages (phages integrated into bacterial genomes) were detected within the active viral fraction. I found that many of these induced prophages demonstrate polyvalent lytic activity, likely implicating induction of viruses as an important process modulating bacterial communities. And finally, I examined the role of an immune effector expressed in the gut of protochordates, like *Ciona*. This protein, known as variable Ig-like chitin-binding protein (VCBP) was shown to differentially influence biofilm formation among different bacterial isolates of the gut. Importantly, the role of this protein in modulating bacterial communities appears to intersect the activity and function of some phages. My dissertation research made an important contribution to the symbiosis field by developing extending the *Ciona* model system into this emerging field, as a new system in which novel aspects of host-microbe interactions can be defined. *Ciona* is a relevant and tractable model for studying trans-kingdom interactions among animal immunity, bacteria and phages during colonization of the gut epithelium.

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